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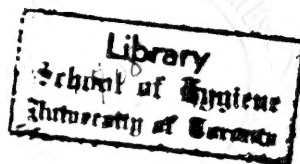
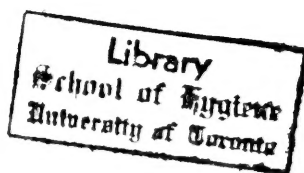
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STUDIES IN ANAPHYLAXIS¹

XIV. ON THE RELATION BETWEEN PRECIPITIN AND SENSITIZIN

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In the following papers the term "Anaphylactic antibody" has been replaced by the word "Sensitizin." This has the advantage of brevity. The word is formed on the analogy of the words precipitin and agglutinin, and carries its own significance—namely that substance which confers sensitization. No distinctions are at present known between the substance responsible for active, and that for passive sensitization, so that both are covered by this term. Besredka some years ago suggested "Sensibilisin," but this is foreign to the spirit of the English tongue, being constructed on the root of the French verb "Sensibiliser" (to sensitize). "Anaphylactin," a term suggested by Anderson, had been used in a different sense by Gay and Southard, which seems to preclude its adoption.

Friedberger (5) was among the first to maintain the identity of precipitin and sensitizin, basing his belief on the fairly constant ratio between these two substances in various sera. Doerr and Russ (1) independently reached the same conclusion on the basis of a similar argument. This relationship, however, does not seem to be uniform, inasmuch as various observers have noted marked departures therefrom. Kraus and Novotny (6) after a careful experimental study of the conditions, categorically deny the identity of precipitin and sensitizin, and decline to accept the foregoing argument based on parallel ratios.

¹The previous studies in this series were published in the *Journal of Medical Research*, 1913-1915.

The most recent attempt to determine the relations between precipitin and sensitizin was undertaken in 1914 by Lake, Osborne and Wells (7). They found that: "At the time the precipitin reaction appears, the passive anaphylactic condition usually can be induced in guinea pigs injected with this precipitating serum." They state their conclusions very cautiously as follows: "Inasmuch as the antisera which gave the precipitin test also caused passive anaphylaxis, it is possible that one and the same antibody is common to these two reactions." Doerr (2) summarizes the situation in the statement that the majority of workers tend to assume three different antibodies, in conformity with the three functions of precipitation, complement fixation, and sensitization.

Recent studies in the immunological aspect of human pneumonia seemed to me to lend renewed interest to this problem. Torrey and Weil (8) found that sensitizin was present during the disease, and generally absent after the crisis, whereas most observers have reported exactly the reverse of the occurrence of agglutinins, which generally appear only after the crisis. The question naturally arises as to the relationship of these two sorts of antibody, both to one another and to the evolution of the disease process. The following experiments seem to shed some light upon the latter question, and at the same time upon the long standing discussion concerning the identity of precipitin and sensitizin.

IDENTITY OF PRECIPITIN AND SENSITIZIN

There is one experiment, which does not seem to have been previously performed, that might bring the issue as regards the identity of precipitin and sensitizin nearer to a decision. If one could make use of the precipitate formed by the union of antigen and precipitin, to sensitize an animal passively, this result might be taken to indicate that precipitin may also act as sensitizin. After a considerable number of ineffectual attempts, it was found possible to accomplish this, as is evident from the following experiments.

Experiment 1. In this experiment the precipitin was supplied by the serum of rabbit 894, which had received repeated injections of horse serum. This animal was bled on June 11. On this date 0.1 cc. of the serum gave a heavy precipitate with 0.01 cc. of horse serum. Its sensitizing value is indicated by the following tests:

- Guinea pig 852.* June 11, 0.1 cc. 894 intraperitoneally.
 June 12, 0.3 cc. horse serum intravenously. Moderate symptoms.
- Guinea pig 853.* June 11, 0.3 cc. 894 intraperitoneally.
 June 12, 0.3 cc. horse serum intravenously. Immediate death.

On June 12 a series of tubes, each containing 1.5 cc. of Serum 894, was mixed with descending amounts of horse serum. The tubes were incubated for one hour, and then kept in the ice-box for forty-eight hours. The precipitation, which occurred almost immediately after mixing, is indicated in the following table:

TABLE I
Serum 894, 1.5 cc.

	HORSE SERUM	PRECIPITATE
	cc.	
(1)	0.3	+++
(2)	0.15	+++
(3)	0.05	++
(4)	0.02	+

The tubes were centrifuged and the supernatant fluid poured off. Five cubic centimeters of salt solution were added to the precipitate, and the tubes were again centrifuged. After pouring off the salt solution, the washed precipitate, which was considered to be practically free from all but traces of the original Serum 894, was employed in the following manner: To each tube was added 2 cc. of salt solution, and the precipitate thoroughly shaken up with it. On June 14 the mixture in each tube was injected intraperitoneally into a guinea pig of about

300 grams weight. Four days later each of these pigs received an intravenous injection of 0.5 cc. of horse serum. These details and the results are indicated in the following table:

TABLE II
Passive sensitization by washed precipitate

	WASHED PRECIPITATE OF SERUM 894, 1.5 CC. AND HORSE SERUM	GUINEA PIG	HORSE SERUM	SYMPTOMS
	cc.		cc.	
(1)	0.3	1	0.5	None
(2)	0.15	2	0.5	Very mild
(3)	0.05	3	0.5	Moderate
(4)	0.02	4	0.5	Death immediate

This table shows that a precipitate produced by the mixture of certain proportions of precipitinogen (horse serum) and precipitin (Serum 894) was competent to produce very effective passive sensitization of the guinea pig. Precipitates produced by a larger proportion of the horse serum were found to be decreasingly effective, while the first animal of the series, which receives the precipitate from the mixture in which the proportion of horse serum to precipitin was the largest, failed to give any evidence of sensitization.

In the preceding experiment 1.5 cc. of Serum 894 had been used, an amount which represented for this serum at least five sensitizing doses, as indicated by the preliminary sensitization tests previously detailed. In a second experiment the same serum was used in exactly the same manner, except that the amount was reduced to 0.5 cc. Horse serum was added in amounts proportional to those used before. The resulting precipitates were washed and injected into guinea pigs, which were tested four days later by the intravenous injection of horse serum. The results were as follows:

TABLE III

Passive sensitization by washed precipitate

	SERUM 894, 0.5 CC. AND HORSE SERUM	GUINEA PIG	HORSE SERUM	RESULT
	cc.		cc.	
(1)	0.1	1	0.3	No symptoms
(2)	0.05	2	0.3	No symptoms
(3)	0.018	3	0.3	No symptoms
(4)	0.007	4	0.3	Severe symptoms (convulsions)

These results indicate that not only the relative proportions of precipitin and precipitinogen, but also the absolute amount of precipitin, is a factor of importance in determining the capacity of a precipitate to induce passive sensitization.

The following table indicates a similar set of results, the minimal sensitizing dose of this rabbit serum being 0.5 cc. The processes in the series were exactly the same as those previously described.

TABLE IV

Passive sensitization by washed precipitate

IMMUNE RABBIT SERUM 190	HORSE SERUM	GUINEA PIG	HORSE SERUM	RESULTS
cc.	cc.		cc.	
2.0	0.2	1	0.3	No symptoms
2.0	0.02	2	0.3	Moderate symptoms
2.0	0.007	3	0.3	Very severe symptoms
1.0	0.02	4	0.3	Very mild symptoms
1.0	0.01	5	0.3	Very mild symptoms
0.8	0.001	6	0.4	Very severe symptoms
0.5	0.001	7	0.4	Moderate symptoms

In spite of the fact that these results seemed fairly conclusive, it was deemed advisable to carry the experiment one step further. Horse serum is a mixture of a number of antigenic substances, albumins and globulins, and this is a factor which might possibly in some unknown manner vitiate the results of the experiment. Crystalline egg albumen, therefore, was prepared by Pinkuss'

method, being precipitated from solution three times. For this material I am indebted to Dr. A. F. Coca.

Experiment 2. Crystalline egg albumen was used to immunize rabbits 882 and 883. The serum of 882 sensitized guinea pigs passively in amounts of 0.1 cc., but not of 0.05 cc. The serum of 883 sensitized in amounts of 0.05 cc. Similarly, the precipitation reactions with 5 per cent of crystalline egg albumen showed that 883 was considerably richer than 882 in precipitins. These sera were employed to make precipitates with graded amounts of crystalline egg albumen, as in Experiment 1. The precipitates, after being washed twice, were injected into a series of guinea pigs, which were tested three days later by the intravenous injection of the egg albumen. The results are indicated in the following table:

TABLE V
Passive sensitization by washed precipitate

IMMUNE SERUM	CRYSTAL- LINE EGG ALBUMEN	PRECIPITATE	GUINEA PIG	EGG ALBUMEN	SYMPTOMS
cc.	cc.			cc.	
<i>Serum 882:</i>					
1	0.01	Slight	1	0.5	None
(10 sens. doses)					
1	0.001	++	2	0.5	Death at once
1	0.0001	Slight	3	0.5	None
<i>Serum 883:</i>					
1	0.1	None or very slight	4	0.3	Moderate
(20 sens. doses)					
1	0.01	+++	5	0.5	Death at once
1	0.001	++	6	0.4	Death at once
1	0.0,001	+	7	0.4	Mild
1	0.00,001	Slight	8	0.4	None

The fourth line in this table represents the so-called prozone in which excess of precipitinogen inhibits precipitation. Here the entire contents of the tube were injected.

A study of this table not only confirms, but very much strengthens the conclusions already reached. Precipitates pro-

duced by mixtures of precipitin and precipitinogen in proper proportions effectively sensitize guinea pigs passively towards the antigen. If a constant amount of immune serum be used, it is found that on each side of a zone of optimum proportions of antigen, lies a zone in which either an excess or a deficiency of antigen decreases the sensitizing value of the resulting precipitate. The explanation of this fact seems evident. An excess of antigen leads to incomplete precipitation of the antibody, part of which remains in solution, as in the prozone. A deficiency of antigen fails to carry down enough precipitate to sensitize. That this explanation is correct will be clear from the evidence submitted in a subsequent article.

The objection may arise that the sensitizing substance although carried down by the precipitate is not actually identical with the precipitin. The association might conceivably be purely mechanical, in other words the sensitizin might be carried down mechanically with the precipitate. In order to test this hypothesis, the following experiments were performed: One cubic centimeter of the serum of a rabbit immunized against horse serum (S. 894) was added to two sensitizing doses (0.1 cc.) of Serum 883 (rabbit versus egg albumen), and again to twenty sensitizing doses (1 cc.) of Serum 883. To each of these two mixtures 0.1 cc. of horse serum was added. Heavy precipitation occurred, owing to the reaction between 894 and horse serum. The supernatant fluid of the first tube was injected into a guinea pig, which responded fatally two days later to the intravenous injection of egg albumen. This shows that no appreciable amount of the sensitizing substances in Serum 883 had been mechanically carried down in the precipitate. The second tube was centrifuged, and the washed precipitate injected into a guinea pig. This guinea pig, when tested four days later by the intravenous injection of egg albumen, displayed no symptoms. This, again, shows that the washed precipitate failed to carry the sensitizing substances of Serum 883. The reverse experiment, in which Serum 894 was mixed with precipitating amounts of Serum 883 and egg albumen, had an identical outcome. Thus it seems clear that the mere act of precipitation does not mechanically carry down

the sensitizing substance. The conclusion seems unavoidable that the latter is identical with the precipitin. In any event, the burden of experimental proof certainly rests with those who might dispute this belief.

It has not invariably been found possible to contrive the proportions of precipitinogen and precipitin so as to produce passive sensitization with the precipitate. Thus Serum 775 (rabbit versus horse serum), which had a high precipitating titer, and which sensitized guinea pigs passively in amounts of 0.1 cc. was tested in the following combinations, without success.

TABLE VI

SERUM 775	HORSE SERUM	PRECIPITATE	WASHED PRE- CIPITATE INTO GUINEA PIG	HORSE SERUM INTRAVE- NOUSLY	SYMPTOMS
cc.	cc.			cc.	
0.5	0.5	+	1	0.5	None
0.5	0.01	++	2	0.5	None
0.5	0.002	+	3	0.5	None
1.0	0.1	+	4	0.5	None
1.0	0.01	++	5	0.5	None
1.0	0.001	+	6	0.5	None

The cause of failure with this particular serum is obscure, but was due, I believe, to the fineness of the precipitate, with consequent dissipation on washing.

The mechanism of sensitization by precipitates is discussed in a separate study.

SEPARATION OF THE PRECIPITATING FROM THE SENSITIZING FUNCTION

The demonstration of identity between precipitin and sensitizin does not, however, entirely dispose of the problem which relates to this substance. It might naturally be assumed, as a consequence of this identity, that wherever precipitin can be shown to exist, sensitizin will be present, and vice versa. As a matter of fact, Doerr (3) in his excellent summary of anaphylaxis in Kolle and Wassermann's Handbook, makes the statement

that these two modes of action of an immune serum are similarly affected by a variety of agents. For example, he specifies the fact that both precipitin and sensitizin resist the action of heat at 56°. Unfortunately for the theory, however, these relations are by no means as constant as might be inferred from Doerr's statement. Thus I have found that heating an immune serum may practically destroy the precipitin, while leaving the sensitizin quite intact. This fact is illustrated in the following experiment.

Experiment 3. Rabbit 775, highly immunized against horse serum, yielded a serum which passively sensitized a guinea pig of 300 grams in amounts of 0.1 cc. The precipitin tests showed that 0.1 cc. in 1 cc. of salt solution gave a distinct precipitate with 0.0001 of horse serum; with larger amounts of horse serum the precipitate was voluminous. One cubic centimeter of Serum 775, added to nine parts of 0.8 per cent salt solution, was heated for one-half hour at 70°. Precipitin tests with 3 cc. of this heated mixture failed to give any reaction with either 0.1, 0.01, or 0.001 of horse serum, though the tubes were observed several days. That the mere dilution could not be held responsible for this result is abundantly proven by Experiment 6 of this paper. On the day following inactivation, five guinea pigs were given subcutaneous inoculations of the heated mixture, three receiving 1 cc. each, and the remaining two, each 2 cc. The former group had an amount corresponding to one sensitizing dose, while the latter received two. Two days later all the animals were given intravenous injection of 0.5 cc. of horse serum. The first group showed symptoms of varying intensity, but none either had convulsions, or died. The two pigs in the second group, however, immediately died.

The same experiment was repeated several times, with identical results. Thus it seems that the serum contained sensitizin in only slightly diminished amount, or activity. In other words, heating at 70° had apparently destroyed the precipitin, but had only slightly affected the sensitizin. Apparently this fact demonstrates a striking difference between these two functions of the serum, and seems to argue for two separate substances, of which

one is thermolabile (precipitin), while the other is thermostable (sensitizin). It seems impossible at first sight to reconcile this observation with the fact that washed precipitate passively sensitizes guinea pigs.

An explanation, however, is quite possible, in keeping with the data of immunology previously ascertained and accepted. It is well known, from the work of Eisenberg and others, that heated precipitin, although it has lost the power of precipitating in the presence of antigen, still retains the capacity of uniting actively with the latter substance. Hence it is usually described as precipitoid or precipitinoid. In the terms of Ehrlich's hypothesis, it is said to have lost its ergophore or zymophore group, while it still retains its haptophore group. Now it is only necessary to assume that only the haptophore group need be present in order to sensitize a second animal passively. In offering this explanation, I wish simply to indicate the analogy of the new facts with those previously ascertained, without necessarily accepting the underlying theory.

Anaphylaxis, according to the theory which I have been led by the observations of numerous experiments to support, consists essentially in the interaction between anchored, or cellular, antibody, and freshly introduced antigen. For this reaction to supervene, it may reasonably be supposed that only the presence of the haptophore group of the antibody is necessary. In passive sensitization, the cells of the body appropriate the introduced antibody, and its characteristic haptophore group remains intact. With the injection of the specific antigen, the haptophore group of the cellular antibody seizes upon the latter, and as a result a cellular response, which constitutes the anaphylactic reaction, supervenes. Thus it becomes evident that the same immune substance may act either as precipitin or as sensitizin in its native state, whereas after heating it would lose the previous function, and retain only the latter. The effect of heat is, practically, to produce a new type of antibody which will sensitize while it does not precipitate. This fact suggests further reflections upon the identity of sensitizin and precipitin. It seems quite possible that the immunized animal may normally

produce antibodies of this same type, which have a haptophore, but no ergophore group. At present, one can only say that antibodies of this type do, indeed, occur; in fact this structure is supposed to be characteristic of the so called first order of antibodies of Ehrlich—the antitoxines. So far as is known, the latter have no sensitizing value, so that at present no sensitizing antibody without an ergophore group is known to exist normally.

There are certain other conditions under which it is possible to determine the presence of sensitizins, although precipitins can not be demonstrated. These observations are described in the following experiments.

Experiment 4. It is a well known fact that precipitating sera that give a voluminous precipitate with certain concentrations

TABLE VII

SERUM 894, 0.1 cc.	PRECIPITATE
Horse serum, 0.01 cc.....	+
Horse serum, 0.1 cc.....	Slight
Horse serum, 1.0 cc.....	—

of the antigen, may fail to give any precipitate with still higher concentrations of the antigen. This zone of absent precipitin reaction is sometimes described as the prozone. Its significance is not entirely clear. Eisenberg (4) believed that the precipitin is soluble in an excess of precipitinogen; others have maintained that the union of precipitinogen with precipitin produces a precipitate only when the latter factor is present in a certain excess. At all events, Eisenberg succeeded in demonstrating that the precipitin is firmly united to the precipitinogen in the fluid of the prozone. The limits of the prozone vary strikingly with various precipitinogens. In the case of horse serum, and of the serum of rabbits immunized thereto, the prozone may in some instances be manifest only when the antigen is present in excess of the antibody.

The addition of further antigen to the prozone mixture naturally produces no precipitation. If the prozone mixture in the above instance be injected into a guinea pig, passive sensitiza-

tion is not induced, owing to the fact that the great excess of antigen desensitizes the animal.

In the case of crystalline egg albumen, however, the relations as regards the prozone are entirely different, as shown by the following table:

TABLE VIII

SERUM 883, 1 cc.	PRECIPITATE
5 per cent crystalline egg albumen, 0.001 cc.....	++
5 per cent crystalline egg albumen, 0.01 cc.....	+
5 per cent crystalline egg albumen, 0.05 cc.....	-

Here the prozone is present in a combination in which the proportion of antigen to antibody is much lower than that shown in Table VII. Furthermore, experiment shows that 0.05 cc. of egg albumen does not completely desensitize a pig which has received 1 cc. of Serum 883. If, now, the prozone mixture here described be injected into a guinea pig, the latter becomes passively sensitized thereby, and after two days responds to an intravenous injection of antigen with marked and unmistakable anaphylactic symptoms, such as paralysis and dyspnoea, although death does not occur. Here, then, is another instance in which a fluid apparently contains sensitizin, but no precipitin.

Finally, one more illustration may be given of a condition in which a fluid may show no precipitin, although sensitizin is readily demonstrable. In this case the sodium carbonate extract of a precipitate is the fluid to be studied.

Experiment 5. In four separate tubes, 1 cc. of Serum 775 (rabbit versus horse serum) was added to 0.1 cc. of horse serum. On the following day, the resulting precipitates were washed twice in salt solution. To the washed precipitate in each of the tubes was added 1 cc. of 1 per cent sodium carbonate in water, the mixture being gently shaken. These tubes were incubated for one-half hour at 40°, according to the method of Gay and Chickering. The mixtures were then centrifuged and the supernatant fluid tested as follows: The supernatant fluid in two of the tubes were tested respectively against 0.01 and 0.001 of

horse serum, and failed to present any precipitation. The fluid from each of the other tubes was injected separately into two guinea pigs. Two days later these animals received an intravenous injection of 0.5 cc. of horse serum; both died in anaphylactic convulsions.

Here again it is clear that the so-called heat carbonate extract of the precipitate apparently possessed no precipitin, yet was amply supplied with sensitizin. The addition of fresh precipitin (Serum 775) to the heat carbonate extract produces a heavy precipitate. This demonstrates the presence of antigen (horse serum) in the extract. It might be assumed, therefore, that antigen and antibody are present in the extract in such proportions as to constitute a prozone. If this were the case, however, sensitization could not be induced, for the reason already given, namely that in the case of horse serum the prozone contains so much antigen as to preclude the possibility of passive sensitization. The constitution of the heat carbonate extract is not definitely known, but it seems clear that the antibody must be present in far larger proportion than in the prozone, while its precipitin, or zymophore, group, has been thrown out of action. The case is, therefore, analogous in some respects to the production of precipitoids by heat.

Thus by three different methods it is possible to produce a fluid in which the specific precipitins are apparently lost, while the sensitizins remain intact. In each of these instances the facts indicate that the haptophore group of the precipitin remains in part or in whole available, while the zymophore group is either neutralized or destroyed.

It remains to mention yet one more condition in which fluid containing precipitin shows marked diminution in its precipitating properties, while its sensitizing value remains intact. And this condition deserves special consideration, not only for the reason that it differs essentially in character from these already described, but also because it seems to offer a reasonable analogy with the conditions which may obtain during an infectious disease. Eisenberg, in his remarkable studies in precipitation, mentions the fact that exactly the same amounts of

precipitin and of precipitinogen which produce marked precipitation in the presence of relatively small amounts of a diluent, such as salt solution, fail completely to give a reaction when diluted with larger amounts. The following experiment illustrates the bearing of this fact on the question at issue.

Experiment 6.

Serum 775 (rabbit vs. horse serum).....	0.1 cc.
Horse serum.....	0.01 cc.
0.8 per cent salt solution.....	1.0 cc.

Immediate flocculent precipitate.

Serum 775.....	0.1 cc.
Horse serum.....	0.01 cc.
0.8 per cent salt solution.....	10.0 cc.

No precipitation after incubation for one hour.

Complete precipitation after forty-eight hours in ice chest.

Although I am unable to confirm Eisenberg's belief that dilution inhibits precipitation inasmuch as it merely delays it, nevertheless within the ordinary limits of observation dilution would materially effect the results.

Serum 775, 0.1 cc. in 1 cc. salt solution, injected into a guinea pig. Three days later this animal received an intravenous injection of 0.5 cc. of horse serum. Immediate death.

The same dose of Serum 775, diluted with 10 cc. of salt solution, were injected into another guinea pig, of about the same size. The subsequent anaphylactic injection likewise produced immediate death.

In this experiment it is evident that mere dilution of the antibody serves markedly to delay precipitation of antigen. On the other hand, passive sensitization is in no wise affected by the dilution. The mechanism of the inhibition exerted by dilution upon precipitation is not entirely understood, but is believed to be physico-chemical in nature. Sensitization, however, depends simply on the absorption and appropriation of the antibody by cells.

The fact that precipitating antibody also possesses the func-

tion of passive sensitization, still leaves two questions open for discussion. In the first place, is all of the precipitating substance identical with sensitizin, or does a part of it exercise a precipitating, but not a sensitizing function? This is a question which can be answered only indirectly. Doerr states that in immune rabbit's serum the precipitating value is a reliable index of the sensitizing titer; in fact, he says the one can be predicted from the other. My own experience largely confirms this statement. On the theory of two different functions of precipitin, of which one alone exercises sensitizing functions, such parallelism seems almost impossible to explain. Moreover, there is no experimental ground for assuming such a condition. It may, therefore, be accepted that all precipitating antibody is also sensitizin. The second question is the reverse of the above: Is all sensitizing antibody capable of producing precipitation. Taken strictly, this question is to be answered in the negative, inasmuch as hemolysins may also act as sensitizins. There the lytic function appears to have replaced that of precipitation. But even on the broader basis it is probable that sensitizin may exist which entirely lacks an ergophore group. The fact that dissociation of precipitate in the test tube, as by sodium carbonate, leads to the production of an antibody which has these properties naturally leads to the expectation that dissociation within the body might lead to similar results. But at the present time our knowledge on the subject is so fragmentary that it is wiser to forego further speculation. At all events, the possibility of this factor should not be forgotten in considering the assertion that in some sera, especially in certain species, the sensitizin and the precipitin do not present parallel curves.

SUMMARY OF EXPERIMENTS

Identity of precipitin with sensitizin

1. Precipitates produced by a combination of horse serum and of the serum of a rabbit immunized against horse serum induce passive sensitization towards horse serum when injected intraperitoneally into a guinea pig.

2. The same results hold of precipitates produced by crystalline egg albumen and the serum of a rabbit immunized thereto.

3. In order to be effective in inducing passive sensitization, precipitates must result from mixtures of antigen and antibody in which the proportions between the two factors do not vary outside of certain fairly wide limits. Marked relative excess or deficiency of either factor produces a precipitate which fails to sensitize passively.

4. The sensitizing antibody is not merely adsorbed, or carried down mechanically with the precipitate. The precipitin is identical with the sensitizin.

Relations between precipitating and sensitizing functions of the antibody

5. If an immune serum be heated at 70° for half an hour, it loses its precipitating power, while its sensitizing value is but slightly impaired.

6. The admixture of antigen in excess inhibits precipitation, but affects sensitization in much slighter degree. The prozone fluid affords a striking illustration of this fact.

7. The heat carbonate extract sensitizes, but has no precipitating effect upon antigen.

8. High dilution of the antibody may markedly delay the precipitin reaction, while not affecting passive sensitization.

9. These data show that various agencies and conditions which inhibit precipitation may impair sensitization to only a moderate, or very slight degree.

THEORETICAL CONCLUSIONS

1. The precipitating substance of immune sera is competent to sensitize guinea pigs passively. In other words, precipitin is also sensitizin. It is conceivable, but improbable, that there may be a fraction of precipitin which lacks the sensitizing function.

2. Antibody may be deprived of its precipitating function by heat without suffering a very material diminution in its

sensitizing value. This observation corresponds with the previously known fact that "precipitoid," or heated precipitin, has retained its combining power with antigen, although it has lost its precipitating power. The precipitating, or ergophore group, is said to be thermolabile; the sensitizing, or haptophore group, to be thermostable.

3. Only the combining (or haptophore) group is essential to passive sensitization. Anaphylaxis therefore consists simply in the cellular reaction due to the fixation of antigen by cellular antibody. These new data therefore confirm and establish the theory of anaphylaxis supported in previous studies of this series.

4. The fact that the coexistence of antigen in the same fluid may inhibit the precipitating power of antibody, while only partially interfering with the sensitizing function, as in the prozone experiments, may explain the divergence in the literature between those who maintain that precipitin and sensitizin run parallel in immune sera and those who deny this relationship.

5. The phenomenon which has been described as dissociation of the precipitate, which probably occurs within the body, and which may be imitated by various laboratory procedures, such as extraction by sodium carbonate, sets free antibody in a form which sensitizes passively but fails to give the precipitin reaction. Such a factor, likewise, would upset the normal parallelism between sensitizin and precipitin.

6. The foregoing consideration may serve to explain the fact that the presence of antibodies may be demonstrated by means of passive sensitization in spite of the fact that the test tube reactions, such as agglutination and precipitation, prove ineffective. In infectious disease the co-existence of the antigen (the infectious agent or its product) in the blood might be expected to produce this result.

The terms ergophore and haptophore group in this paper are used merely as a convenient mode of expression, and without any theoretical implication.

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STUDIES IN ANAPHYLAXIS

XV. EQUILIBRIUM IN PRECIPITATION REACTIONS. EQUILIBRIUM IN COMBINATION

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INTRODUCTION

Interest in the equilibrium of precipitation reactions is now about fifteen years old, and dates from certain observations made in 1902 by Linossier and Lemoine, and by Eisenberg. Linossier and Lemoine (9) mixed antigen and precipitin in the test-tube, and, after removing the resulting precipitate, again tested the supernatant fluid for the presence of each factor. For example, horse serum, and the serum of a rabbit immunized thereto, were mixed in various proportions; the supernatant fluids in each tube were then divided into two equal portions, and to the one was added horse serum, and to the other the immune rabbit serum. If precipitation resulted in the former, it indicated the presence of remnants of precipitin; if in the latter, it showed that a fraction of the original horse serum still remained in the supernatant fluid. As a matter of fact, Eisenberg (6, 7) found that both substances could practically always be demonstrated. He summarized this observation in the following words:

In every precipitin reaction, in addition to the reaction product, the precipitate, a fraction of each of the reagents is demonstrable in the supernatant fluid. These reactive substances coexist in the fluid without combining ("reaktionslos"). In accordance with a general law of chemistry, an equilibrium has become established between the

two components of the system, and only by the addition of fresh increments of either reagent can this equilibrium be disturbed, leading to a renewal of the reaction. An excess of either factor in the original mixture may reduce the amount of the other which persists in the supernatant fluid to a minute remnant, yet by proper methods even that remnant can be detected.

These observations have been amply confirmed by subsequent workers.

From the phenomena above described, the deduction was drawn that precipitating antibody and antigen may coexist both in the test-tube, and in the blood of immunized animals, without entering into combination, and without undergoing precipitation. This inference has been seriously questioned by von Dungern (4). In the first place, he repeated the experiment of Eisenberg, using as antigen the blood plasma of various crustacea and molluscs; the antibody was furnished by an immune rabbit serum. Over a long series of tests, he found that the supernatant fluid, after removal of the precipitate, could hardly ever be shown to contain both antigen and antibody, provided that the tests were made with the same stock of antigen and antibody as entered into the original reaction. This certainly destroys the universality of Eisenberg's law, and leads to the quest for some special factor to explain his results. This factor v. Dungern supplies in his theory of a multiplicity of antigens in the same antigenic serum. According to this view, horse serum must be regarded not as a single substance, but as a complex of substances, some of which are probably rapid and active stimulators of antibody formation, while others are the reverse. The serum of an immune rabbit, therefore, would contain many antibodies to a certain component of horse serum (partial antigen I), and but few to a second (partial antigen II). If the horse serum is mixed with the immune serum, it will be very likely to happen that antibody I may be in excess, so that after precipitation of all of its own antigen, part of it still remains in the supernatant fluid. On the other hand, the reverse may be true of antibody II, so that in this instance part of antigen II remains in the supernatant fluid. On this assumption, the addi-

tion either of fresh horse serum or of fresh antibody would produce additional precipitation. Thus, it is quite clear that the phenomenon observed by Eisenberg is susceptible of an entirely different interpretation from his own, and that it is not necessary to conclude that antigen and homologous antibody coexist in the supernatant fluid after precipitation. The fact that such a multiplicity of antigens does exist in the same antigenic serum is in itself unquestionable. Coca and I (11) found that if egg albumin and egg globulin were separated by chemical means, and different rabbits were immunized to each substance, the resulting immune sera reacted powerfully each with its own antigen, and very weakly with the other antigen. Ascoli (1) reached a similar conclusion. The fact that von Dungern's reactions resulted so differently from Eisenberg's is possibly attributable to the fact that the antigens employed by the former were fairly simple in their constitution.

In the same paper, von Dungern (4) recorded the observation that two antisera to the same antigen might precipitate when mixed, a fact which has been repeatedly confirmed, notably by Uhlenhuth and Weidanz (10). This observation of v. Dungern's may be illustrated as follows. Two rabbits are immunized by an identical series of injections against horse serum, the one being bled five days after the last injection of antigen, and the other ten days. The first rabbit yields a serum which gives a distinct precipitate when added to dilutions of horse serum; in other words, it contains precipitin for horse serum. The serum of the second rabbit, we will assume, gives a considerably higher titer for precipitins than the first. Moreover, if added to the first serum, it will produce a precipitate therewith; this fact is taken to demonstrate the presence of precipitinogen, of remnants of horse serum, in the serum of the first rabbit. In other words, the serum of the first rabbit has been shown to contain not alone precipitin, but also remnants of antigen, and these two substances, although possessing ordinarily a high degree of affinity for one another, are here supposed to exist side by side in the immune serum in ununited, unprecipitated form. Von Dungern interpreted this phenomenon in the same terms as the test tube

phenomenon, ascribing it to the presence of multiple antigens. By others, however, including Uhlenhuth (10), the theory of equilibrium between antigen and antibody was invoked in explanation, just as Eisenberg had invoked it for the test tube reactions.

Gay and Rusk (5) observed the fact that if horse serum were injected into a rabbit immunized thereto, both antigen and antibody could be demonstrated in the blood of the animal for a short period of time. This experiment presents the same conditions and the same problems as those already described.

There is only one additional fact of interest which has been added to these already described. Uhlenhuth (10) states that in immune sera the phenomenon of auto-precipitation may at times occur. Auto-precipitation denotes the spontaneous formation of a precipitate in an immune serum, and it might be interpreted to mean that the ununited antigen and antibody had gradually combined, leading to the formation of a precipitate. Such a conclusion, however, is not necessarily correct. As Uhlenhuth has himself pointed out, normal sera also not infrequently present auto-precipitation, and in this instance there can be no question of a union between antigen and antibody. Zinsser and Young (13) attempted to prove that auto-precipitates in immune sera were antigen-antibody complexes, by showing that they fix complement. Unfortunately, the spontaneous precipitates of normal sera also fix or absorb complement, as I have found in three such precipitates which I studied. There is at present no argument, and no experimental evidence, which would lead one to accept as satisfactorily proven either the theory of Eisenberg or that of von Dungern. In view of this situation, it seems almost superfluous to examine into the explanations which have been advanced to account for the supposed coexistence of antigen and antibody in the same fluid. Zinsser (12) criticises the analogy of mass action and equilibrium, as advanced by Eisenberg, on the ground that "it has not been experimentally shown that colloidal substances react in accordance with the laws of mass action as observed for simpler chemical substances." He then attempts to establish the fact that antigen and antibody

do actually coexist, by showing that a third colloid, such as gum arabic, may serve to prevent the interaction of two mutually precipitating colloids, such as gelatin and arsenic trisulphide. In Zinsser's own words, it may certainly be said that it has not been shown that antigen and antibody react according to the laws governing such colloids as arsenic sulphide, and so forth. Indeed, Ehrlich (5) himself protested most vigorously against arguments based on this type of analogy, and Bordet (2), perhaps the most successful of those who have pursued this line of thought, specifically states in his last general critique that "although it is very probable that antibodies are really colloids, we cannot with certainty say as much of the antigens."

After all, however, the problem cannot be settled by analogies; it is essentially a matter in which only a demonstration of the facts will put an end to the present divergence of opinion.

Before passing to the experimental part of this paper, it may not be amiss to say a further word concerning auto-precipitation. It is, indeed, true that freshly drawn immune serum may present precipitation within a period of seventy-two hours, and it is very likely that this precipitation is due to the union of antigen and antibody. The cells of the body are constantly discharging antibodies into the circulation, and until all the circulating antigen is neutralized, each fresh increment of antibody will gradually unite with a fraction of antigen. But it is not to be supposed that this union is instantaneous. Everyone who has watched precipitation reactions, especially if performed in high dilution, is aware of the fact that some of the precipitate forms instantaneously, while the rest of it may gradually accumulate over a period of several hours. Indeed, Müller has divided precipitins into those of high and of low avidity. It is to be expected that part of the antibody which entered the blood just prior to bleeding, may have a low avidity, and may not enter into combination with antigen for several hours, or even days, after drawing. This, however, is not what is meant by the coexistence of antigen and antibody. The phenomena observed by Linossier and Lemoine and by Eisenberg are not diminished in intensity by prolonged keeping of the serums or of the super-

natant fluids which enter into these reactions. In other words, the antigen and the antibody, if such they be, remain permanently disunited. Unfortunately, this very important distinction has not always been recognized in the literature. The distinction may be grasped from the following illustration. If a rabbit is bled four or five days after receiving a large dose of antigen, auto-precipitation may at once begin, and may continue for two or three days. This phenomenon represents the union of ununited antibody with the antigen in the blood. After three days, however, this process is at an end. For an indefinite period, however, it is still possible to carry out the experiment previously described, namely, to demonstrate the presence of antigen in the serum by the addition of an homologous antiserum of a still higher titer. The problem at issue is not concerned with the immediate auto-precipitation, the significance of which is hardly open to doubt. It is concerned with the character of the antigen and antibody which persist in the serum, side by side, for an indefinite period. The question remains: Are these two substances related as specific antigen and antibody, or as antigen I and antibody II?

EXPERIMENTAL STUDIES

The observations of Eisenberg were made with horse serum and with the serum of rabbits immunized thereto. In view of the fact that his paper presents no tables of his results, it seemed advisable to repeat his experiment. The horse serum and rabbit serum were mixed in varying proportions, and after twenty-four or forty-eight hours the resulting precipitate was removed by centrifugation. The following tables represent the precipitation obtained in testing portions of the supernatant fluid by the addition respectively, of antigen and antibody. In making the latter tests, a portion of the supernatant fluid was regularly kept as a control.

The experiments were carried out with three different immune sera, and the conditions were quantitatively so varied, as to test the truth of Eisenberg's observation over a wide range of

conditions. The tables demonstrate a remarkable uniformity in the results obtained. In the first place, they show a wide mid-zone; here, the mixture of antigen and antibody yields a

TABLE I
Horse serum as precipitinogen

SERUM 894 RABBIT VERSUS HORSE SERUM	HORSE SERUM	PRECIPITATE AFTER FORTY-EIGHT HOURS	SUPERNATANT FLUID	
			+0.01 cc. horse serum	+0.1 serum 894
cc.	cc.			
0.15	0.03	++	No precipitation	++
0.15	0.015	++	Slight	++
0.15	0.005	+	+	+
0.15	0.002	+	++	Slight

TABLE II
Horse serum as precipitinogen

SERUM 802 RABBIT VERSUS HORSE SERUM	HORSE SERUM	PRECIPITATE AFTER TWENTY-FOUR HOURS	SUPERNATANT FLUID	
			+ 0.01 cc. horse serum	+ 0.25 cc. serum 802
cc.	cc.			
1	0.2	++++	—	++
1	0.1	+++	—	++
1	0.05	++	Slight	++
1	0.01	+	+	+

TABLE III
Horse serum as precipitinogen

SERUM 775 RABBIT VERSUS HORSE SERUM	HORSE SERUM	PRECIPITATE AFTER TWENTY-FOUR HOURS	SUPERNATANT FLUID	
			+ 0.1 cc. horse serum	+ 1 cc. serum 775
cc.	cc.			
1	0.01	++	++	++
1	0.1	+	+	+++

supernatant fluid which contains both antigen and antibody. Second, they show a prozone, in which a relative excess of antigen in the original mixtures yields a supernatant fluid containing antigen, but no antibody. Third, they show a postzone, in which

relative excess of antibody in the original mixtures yields a supernatant fluid containing antibody and only traces of antigen. Thus, although Eisenberg's contention is, in the main, confirmed, it does not appear to be accurately correct to state, as he does, that no matter what the proportions of reagents in the original mixture, it is always possible to demonstrate traces of both of them in the supernatant fluid. This emendation, however, does not materially affect the significance of the observation, nor its apparent conformity with the laws of mass action. It is also of interest to note that the supernatant fluids did not change their reactions even if kept for a week before making the final tests; nor did they in the interval present any further precipitation. In other words, the equilibrium established between antigen and antibody appears to be permanent.

The next step in the analysis was an attempt to arrive at some means of explaining this phenomenon. According to von Dungern's theory, the antigen and the antibody in the supernatant fluid are only accidentally associated, but are not related as specific antigen and antibody. In order to test this view, it seemed essential to secure a homogeneous, chemically pure protein, and to make use of this as antigen. Such a substance is available in the form of crystalline egg albumen, and this was therefore selected for the experiment. It was kindly prepared for me by Dr. Coca by Pinkuss' method. By way of preliminary, however, Eisenberg's experiment was also carried out with raw egg albumen and the serum of a rabbit immunized thereto. The results are shown in Table IV.

TABLE IV
Raw egg albumen as precipitinogen

SERUM 13 RABBIT VERSUS RAW EGG ALBUMEN	RAW EGG ALBUMEN	PRECIPITATE AFTER FORTY-EIGHT HOURS	SUPERNATANT FLUID	
			+ 0.005 cc. raw egg albumen	+ 0.5 cc. serum 13
cc.	cc.			
0.5	0.1	++	—	slight
0.5	0.01	++	slight	+
0.5	0.001	++	slight	+
0.5	0.0001	slight	+	++

The results are entirely similar to those obtained in the preceding experiments.

In order to obtain a precipitin for crystalline egg albumen, this substance was given to rabbits in a series of intravenous injections. Five rabbits were employed for this purpose. The resulting antiserum was, in every instance, very high, although the titer of the five sera varied considerably. The best serum was yielded by Rabbit 883. The precipitation values of this serum may be judged from the following table:

TABLE V
Crystalline egg albumen: Titer of antiserum

SERUM 883	5 PER CENT CRYSTALLINE EGG ALBUMEN	PRECIPITATION
cc.		
1	0.1	very slight (prozone)
	0.01	+++
	0.001	++
	0.0001	+
	0.000001	slight
0.5	0.1	—
	0.01	++
	0.001	+++
0.1	0.01	slight
	0.001	+
	0.0001	slight
	0.00001	—

This table shows that 1 cc. of the serum gave marked precipitation with 1/10,000 cc. of 5 per cent egg albumen. The serum sensitized guinea pigs of 250 grams weight, in amounts of 0.05 cc. so that the subsequent intravenous injection of 0.0001 cc. of the egg albumen produced immediate death. With this serum an experiment in equilibrium was carried out in the manner summarized in Table VI.

The table at once reveals a striking difference from the result obtaining in all the previous experiments. In no test does the supernatant fluid appear as giving a reaction for both antigen and antibody. Where the one is present, the other is absent.

At the time that these experiment were made, I was entirely convinced that von Dungern's explanation was incorrect, and I

TABLE VI

Crystalline egg albumen as precipitinogen

SERUM 883	5 PER CENT CRYSTALLINE EGG ALBUMEN	PRECIPITATE	SUPERNATANT FLUID	
			+ 0.001 5 per cent crystal- line egg albumen	+ 0.5 cc. serum 883
cc.				
1	0.1	very slight	—	++
	0.01	+++	—	+
	0.001	++	+	—
	0.0001	+	++	—
	0.00001	slight	+++	—

therefore carried the tests still further, believing that a proper mixture of the two substances could not fail to reveal a state of equilibrium. The table shows that such a mixture would have to contain an amount of antigen lying somewhere between 0.01 cc. and 0.001 cc. Such mixtures were therefore prepared, and the supernatant fluid tested as before. The results are shown in Table VII.

TABLE VII

Crystalline egg albumen as precipitinogen

SERUM 883	5 PER CENT CRYSTALLINE EGG ALBUMEN	PRECIPITATE	SUPERNATANT FLUID	
			+ 0.001 5 per cent crystal- line egg albumen	+ 0.5 cc. serum 883
cc.				
1	0.006	++	—	+
	0.005	++	—	+
	0.004	++	+	—
	0.003	++	+	—

The result of this test is again the same. The amounts of antibody in the original mixture were now varied. The examination of the supernatant fluid was elaborated to such an extent that it was tested against three different amounts of antigen and antibody, respectively. All of these attempts ended in failure. By no possible manipulation of the reagents could it be shown that antigen and antibody ever coexisted in the fluids.

The same methods of study were applied to the other rabbit sera immunized against egg albumen. The results, however, were uniformly the same.

Thus, the conclusion was drawn that when a chemically pure antigen is used, results of the type described by Eisenberg are not obtainable. In other words, antigen and antibody, in this case, cannot coexist without uniting to form precipitate, and this union goes on to the complete exhaustion of either component, leaving an excess of the other alone in the supernatant fluid. This conclusion seems to vindicate von Dungern's theory, and to show that Eisenberg's experiment demonstrates only the coexistence of unrelated antigen and antibody, but not of homologous substances. This view, furthermore, is strongly supported by an additional set of experiments, carried out in the following manner. Assuming it to be true that raw egg albumen is composed of a number of antigens, of which the substance represented by crystalline egg albumen is one, it ought to be possible to exhaust all of its content in crystalline egg albumen by adding an excess of the antiserum to that particular component. The removal of the resulting precipitate should leave a supernatant fluid which would give no further precipitation upon the further addition of the same antiserum, for the reason that its antigen has been completely precipitated out. On the other hand, if it is true that raw egg albumen contains the additional antigens postulated by the theory, it ought to be possible to produce further precipitation in the supernatant fluid of the experiment above described, by adding an antiserum against raw egg albumen, for such an antiserum should contain antibodies to several or all of the component antigens. If the experiment produces a result in conformity with the plan above described, it would seem impossible to escape the theory of a multiplicity of antigens and antibodies, when raw substances, such as horse serum or egg albumen are employed in the immunological experiment. As a matter of fact, the experiment resulted exactly as the forecast suggests.

Experiment. To 0.002 of native egg albumen was added 2 cc. of Serum 775, from a rabbit highly immunized against crystalline egg

albumen; salt solution was added up to 4 cc. After incubation for twenty-four hours, the supernatant fluid was divided into four parts, and tested as follows: To the four tubes were added, respectively, crystalline egg albumen, Serum 775, raw egg albumen, and Serum 13 of a rabbit highly immunized against raw egg albumen. Crystalline egg albumen gave a heavy precipitate; Serum 775 gave no precipitate; Serum 13, and raw egg albumen both gave good precipitates.

An interpretation of these results is possible only on the theory of multiple antigens in raw egg albumen, and of multiple antibodies in the corresponding antiserum. The further addition of Serum 775 gave a negative result for the reason that no more crystalline egg albumen remained in the fluid. Serum 13 gave a positive result because the fluid still contained other partial antigens, like ovoglobulin or ovomucin, and Serum 13 has antibodies in greater or less degree to all of these partial antigens. Crystalline egg albumen and raw egg albumen both gave a precipitate for the reason that an excess of Serum 775 remains in the supernatant fluid, which naturally precipitates with the purified egg albumen, and with the corresponding constituent of raw egg albumen.

The converse of this experiment was also performed. If crystalline egg albumen is added in excess to Serum 13, a precipitate results. The supernatant fluid no longer reacts with crystalline egg albumen, but gives a heavy precipitate with raw egg albumen. Moreover, the addition of Serum 775 and of Serum 13 is also positive. As the previous experiment was designed to demonstrate multiple antigens in native egg albumen, this experiment reveals the presence of multiple antibodies in the corresponding antiserum, Serum 13.

Thus, the contention of v. Dungern that Eisenberg's results are due simply to a multiplicity of antigens, seems to be amply sustained. It still remains to determine whether the mutual precipitation of immune sera, as originally described by von Dungern, is also to be explained upon the same basis. It is conceivable that the conditions present in the circulating blood might hinder the combination of precipitin with precipitinogen, and so permit them to circulate together without uniting. Zinsser

and Young (13) have suggested that the blood might act as a "protective colloid," and so prevent precipitation. In order to put this belief to the test of fact, the following experiment was devised.

Experiment. Crystalline egg albumen, 0.001 cc., was added to each of three test-tubes, containing respectively, 1 cc. of salt solution, 1 cc. of normal guinea-pig serum, and 1 cc. of normal rabbit serum. The tubes were well shaken, and then 1 cc. of Serum 799 added to each. After twenty-four hours, the resulting precipitate was removed by centrifugation, and the supernatant fluids tested by the addition of identical amounts of antigen or antibody. The addition of serum was found to have produced no change in the results; in the case of none of the fluids were both reactions positive. The same experiment was repeated, using 4 cc. of the diluents instead of 1 cc. The results, however, were the same. Thus, as shown by these experiments, it seems certain that in the test-tube, at least, no such thing exists as a protective colloidal action. These results are shown in Table VIII.

TABLE VIII
Protective colloid tested

0.001 CC. EGG ALBUMEN (CRYSTALLINE) 1 CC. SERUM 799	PRECIPITATE	SUPERNATANT FLUID	
		+ 0.001 cc. egg albumen	+ 1 cc. serum 799
1 cc. salt solution.....	++	—	+
1 cc. guinea pig serum.....	++	—	+
1 cc. rabbit serum.....	++	—	+
4 cc. salt solution.....	++	—	+
4 cc. guinea pig serum.....	++	—	+
4 cc. rabbit serum.....	++	—	+

The theory of protective colloidal action appears, therefore, to lack substantiation, and to afford an illustration of the danger of arguments based on the analogy of colloid chemistry. Indeed, Buxton and Teague (3) at the close of one of the most elaborate studies of colloidal flocculation that exists, expressly utter a warning against the premature application of the data of this field of study to the reactions of immunology and point out particularly the difference in the protective action of a third colloid.

The study of the blood itself affords striking support to the view that the apparent coexistence of precipitin and precipitinogen is illusory. In the case of rabbits immunized against horse serum, it is a simple matter to produce precipitation by mixing the various sera. The same is true of sera derived from rabbits immunized against raw egg albumen. In a long series of trials, however, I have never achieved this result by mixing sera derived from rabbits injected with crystalline egg albumen. There seems to be only one explanation available for this fact. In the latter case, there is a homogeneous antigen which is precipitated out of the blood by an excess of homogeneous antibody. In the former, however, the blood of one rabbit may contain precipitin to antigen I, and, at the same time, antigen II may not yet have been neutralized by the antibody production of the animal; in a second animal, these conditions may be reversed, giving antigen I and antibody II together in the blood; naturally the mixture of two such sera will give precipitation.

All of the experiments lead, therefore, to but one result. Under no conditions can precipitin and precipitinogen exist in the same fluid without undergoing union, and producing precipitation. It is, indeed, true that precipitation can be somewhat delayed by very high dilution of the reactive substances, but within a measurably brief period of time the reaction nevertheless goes on to completion. In the light of these results, it is fair to conclude that instances of the apparent coexistence of antigen and antibody, as illustrated by certain experiments in the literature, are actually to be explained upon an altogether different basis, namely, the multiplicity of antigens.

SUMMARY OF EXPERIMENTS

1. When horse serum is mixed in proper proportions with the serum of a rabbit immunized thereto, and the resulting precipitate is removed by centrifugation, the supernatant fluid is found to contain both antigen and antibody, in confirmation of Eisenberg's observations.
2. The same results are obtained with raw egg albumen and the serum of a rabbit immunized thereto.

3. When purified crystalline egg albumen is used as antigen, however, the results are entirely different. If this antigen is mixed in graded proportions with the serum of a rabbit immunized thereto, and the resulting precipitates are removed by centrifugation, the supernatant fluid never contains both antigen and antibody; either one is present alone.

4. It is shown by absorption experiments, using crystalline egg albumen, raw egg albumen, and their respective antisera as factors, that raw egg albumen contains more than one antigen, and that the antiserum thereto correspondingly contains more than one antibody.

5. Protective action by a third colloid, preventing complete interaction of antigen and antibody, has not been demonstrable in the experiments.

6. It has not been found possible to induce cross-precipitation by mixing the sera of different rabbits immunized against crystalline egg albumen. This succeeds, however, when raw egg albumen is the antigen.

THEORETICAL CONCLUSIONS

1. Antigen and precipitating antibody do not coexist in the same fluid without undergoing union and precipitation. The theory that they do so coexist, is fallacious, and is based on the use of a mixed antigenic substance, such as horse serum, which actually contains numerous separate antigenic substances. Similarly, the observation that two similar antisera may give a precipitate when mixed, is due to the fallacy involved in the use of a mixed antigen. Von Dungern's hypothetical analysis of these two phenomena is thus experimentally sustained. Although a third colloid may be effective in preventing the complete union of two mutually precipitating colloids of the character of arsenic trisulfid and gelatin, this condition is not demonstrable in the case of antigen and precipitating antibody. These substances appear to react according to laws proper to themselves, which must be empirically ascertained, and their mode of interaction cannot be foretold by analogy either with the laws of mass action or of colloidal chemistry.

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STUDIES IN ANAPHYLAXIS

XVI. EQUILIBRIUM IN PRECIPITATION REACTIONS.— DISSOCIATION

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INTRODUCTION

As regards the composition of the precipitate, a great deal has been written, and there has been considerable discussion as to whether it is composed wholly of precipitinogen, or wholly of precipitin, or in part of both. That the last named is the correct view of its composition will, I think, be evident from a large number of experiments included in the present study. In order to establish this fact at the outset, however, the following experiment is inserted:

Experiment. To 1 cc. of Serum 894, rabbit versus horse serum, is added 0.02 cc. of horse serum. The resulting precipitate is thoroughly washed, and is then injected intraperitoneally into a guinea pig. Four animals are prepared in this manner. After four days, two of these pigs are given an intravenous injection of 0.5 cc. of horse serum. Both die in anaphylactic convulsions. This demonstrates by the method of passive sensitization the presence of rabbit-versus-horse antibodies in the precipitate. The other pigs are injected with horse serum, after an interval of six weeks, and die in convulsions. In view of the fact that passive sensitization never persists beyond a period of two weeks, these last two reactions can result only from active sensitization towards horse serum. It is inferred, therefore, that horse serum persists in the precipitate. Thus, the precipitate is shown to contain both antigen and antibody.

The condition of equilibrium in which these two constituents of the precipitate exist has been the subject of some study. Eisenberg found that the antigen was in an unsaturated condition, whereas antibody was always saturated. This is a problem which can best be approached after the data on dissociation of the precipitate have been discussed.

By dissociation is meant the disruption of the antigen-antibody complex. Such a complex may be composed of toxin and antitoxin; or of precipitinogen and precipitin in the form of precipitate; or of agglutinin plus agglutinable substance; or of hemolysin fixed by red blood cells. Each of these modes of dissociation has been the subject of experimental study. The application of this study to the present problem is two fold. It has an important bearing, in the first place, on the theoretical aspects of equilibrium in immunology. In the second place, it applies directly to the fact that animals may be passively sensitized with precipitate, suggesting that perhaps within the body the sensitizin is split off from the precipitate, and becomes available for a fresh combination. Finally, from the therapeutic side, the recent work of Chickering indicates that the dissociative treatment of precipitates may prove to be of value in the preparation of therapeutic sera.

In the case of toxin-antitoxin mixtures, very thorough studies by Sachs and others have shown that the complex can be split by heat or chemical measures, yielding both fractions in demonstrable form. The dissociation of hemolysin from red blood cells was studied by Muir, Bordet (1) and others. Muir (4) found that the addition of fresh salt solution to red cells saturated with hemolytic amboceptor sufficed to set free a small part of the antibody. If fresh red cells are added, the dissociation is much greater. The same result has been accomplished in the case of agglutinated bacteria by Landsteiner (3). One of the most interesting features of all this work is the fact that the dissociated antibody appears to retain its original functions: it is still capable of neutralizing toxin, or of hemolyzing red cells, or of agglutinating bacteria. In my own experiments, as will subsequently appear, the dissociated antibody does, indeed, retain its com-

binning affinity for the antigen, but has completely lost the associated function of producing precipitation. In the Ehrlich terminology, it has kept its haptophore group, but has lost its ergophore group. This divergence, however is more striking in the case of dissociated agglutinin than of dissociated hemolysin, for the reason that the hemolytic function of the attached antibody is intentionally restrained in the first stage of the experiment through absence of complement. If complement be added, as Bordet showed, the hemolyzed cells do not give up an effective hemolysin.

Dissociation of precipitin from the precipitate has recently been carefully studied by Chickering (2). He prepared an extract of the pneumococcus, which he then exposed to the action of an antipneumococcus serum. A precipitate was produced. This precipitate was treated in various ways, such as extraction by salt solution, or a 1 per cent solution of sodium carbonate, at a temperature of 40°. The carbonate extract was found to contain agglutinin, precipitin, and protective antibody in amounts which indicated that it had been extracted almost to completion. Chickering tested the extract for precipitinogen and found that none could be demonstrated.

The present study was based primarily on the observation that a guinea pig can be either actively or passively sensitized by means of a precipitate. Hence, it seemed likely that in the living body both factors are dissociable.

METHODS

The methods employed for extraction consisted in the exposure of the precipitate to the action of various fluid, such as salt solution, sodium carbonate solution, trypsin, and a suspension of leucocytes. The determination of the presence of the precipitinogen in the extract offered no difficulty, inasmuch as it could easily be reprecipitated by the addition of fresh precipitin. The demonstration of precipitin in the extract was not so simple. In Chickering's experiments this was evidently accomplished without difficulty simply by the addition of precipitinogen, which again yields a precipitate. In my own experiments, however,

this method has regularly and invariably failed to give a positive result. Hence, it became necessary to attempt the demonstration of the dissociated antibody by other means, namely, the method of passive sensitization. In a previous paper it was shown that precipitin may become so changed that it loses its precipitating function, while still retaining its sensitizing power. It was surmised that such might be the case in dissociation, and the outcome of the experiments has amply justified this expectation.

It is interesting, in this connection, to contrast the result of experiments calculated to determine the presence both of precipitin and of sensitizin in the supernatant fluid remaining after a precipitate has been removed. These experiments yield quantitatively parallel data for these two substances, or rather for these two functions of the antibody. The fact that the extract of the precipitate sensitizes effectively, but does not precipitate, is due to the dissociation of antibody in a form which, to use the current terminology, has retained its haptophore group while it has lost its ergophore function. These facts are illustrated in the following experiment.

Experiment. 1 cc. of the serum of a rabbit immunized against horse serum was added to graded amounts of horse serum. The precipitates were removed by centrifugation, and the supernatant fluids were tested for the presence of antigen and antibody by Eisenberg's method of precipitation. The results showed the presence of both factors in all the fluids; the amount of precipitin remaining was in inverse proportion to the amount of antigen present in the original mixture. A second identical series was prepared, and the supernatant fluids were injected into a series of guinea pigs, which were tested after an interval of three days for passive sensitization. In this series the degree of anaphylactic response in each instance, directly corresponded to the amount of precipitation obtained in the preliminary tests for precipitin (col. III of the table). This shows, like the experiments reported in the first part of this paper, that under normal conditions precipitin and sensitizin are identical. If, now, the precipitates of the present experimental series be treated with 1 per cent sodium carbonate, at a temperature of 40° for one-half hour, and the extracts be tested as above, the results are quite different. The precipitin tests reveal

the presence of antigen in all of the tubes, and of antibody (precipitin) in none. But if guinea pigs be passively sensitized with the extracts, the anaphylactic reactions are pronounced in two of the animals, upon the subsequent injection of the antigen.

The results of the preceding experiment will be clearer in tabulated form.

TABLE I

S. R. \wedge H. S.	HORSE SERUM	PRECIPITATE	SUPERNATANT FLUID			SUPERNATANT FLUID OF CARBONATE EXTRACT		
			Precipitation		Symptoms, passive sensitization	Precipitation		Symptoms, passive sensitiza- tion
			+ 0.01 cc. horse serum	+ 0.5 cc. S. R. > h. s.		+ 0.01 cc. horse serum	+ 0.5 cc. S. R. > h. s.	
cc.	cc.							
1.5	0.3	+	Very slight	++	None	—	++	Mild
	0.15	++	Slight	++	Mild	—	+	Death
	0.05	++	+	+	Moderate	—	+	Severe
	0.02	Slight	++	Slight	Death	—	Very slight	None

Thus, after extraction of a precipitate, the haptophore (or sensitizing) group remains, while the ergophore (or precipitating) group can be longer demonstrated. This result will readily be understood in the light of the preceding papers. The findings do not agree with those of Chickering, from which one would infer that extracted precipitin does not invariably lose its ergophore group. At all events, in the present study in dissociation, the conditions are such as to dictate the use of the method of passive sensitization to determine the presence of free antibody; the precipitation method is not available.

EXTRACTION WITH SALT SOLUTION

Treatment of the precipitate with salt solution in the incubator gives an extract which contains a very small amount of antigen. The precipitin and the anaphylactic reactions alike fail to reveal the presence of antibody. The extracted precipitate, if injected into guinea pigs, is found to have lost little, if any, of its antibody content. Thus, we may say that this method of extraction leads to practically no dissociation.

EXTRACTION WITH SOLUTIONS OF SODIUM CARBONATE

I was led to the use of this substance through the observation that trypsin, which had been rendered alkaline thereby, produced active dissociation. The controls with sodium carbonate alone, however, also gave a pronounced result. After these experiments had been performed, Chickering's observations were published, which showed the carbonate method to be exceedingly effective.

Extraction with carbonate may be performed with solutions of various strength; I have used one per cent, and one-tenth per cent. The extracts contain large amounts of the antigen. The test for precipitin is invariably negative. The method of passive sensitization, however, gives positive reactions. The grade of these reactions is dependent upon the constitution of the original mixtures, as can be seen from the table. If the antigen is in excess, the extract is relatively poor in antibody, for the reason that probably only a portion of the antibody is precipitated, the remainder being held in solution by the excess of antigen, as in the prozone. If the antigen was added in extremely small amounts, the volume of the resulting precipitate is too small to give a good yield of antibody on extraction. Between these two extremes lies a wide zone of optimum proportions. These results are very similar to those obtained in passive sensitization by injection of the precipitate, as described in study XIV of this series. The injection of the precipitate after extraction fails to sensitize passively.

Carbonate extraction, therefore, yields a fluid containing both antigen and antibody, the latter, however, deprived of its precipitating function. These results are not in entire accordance with those recorded by Chickering. He could not demonstrate the presence of antigen in the extract, but did find that precipitin could be demonstrated. The cause of this divergence in findings is at present obscure.

TRYPSIN EXTRACTION

Trypsin was made up in various strengths, ranging from one-tenth to one per cent, and these solutions were rendered alkaline with sodium carbonate. These solutions were used to extract precipitates. The extracts contained both antigen and antibody; moreover, the latter was in the form of precipitin, and reacted with antigen to produce a precipitate.

Dissociation of antigen-antibody complexes with the aid of trypsin was practised by Teruuchi (5) who found that it freed part of the antigen, but no antibody. He made his experiments with cobra venom and anti-cobra serum. The extracts were tested for their toxic and their antitoxic values, respectively.

EXTRACTION BY LEUCOCYTES

Leucocytes were obtained by injecting the pleural cavity of rabbits with aleuronat in salt solution; on the following day the animals were killed, and the supernatant fluid was pipetted off. The leucocytes were washed twice in salt solution before using. When such a leucocyte suspension was added to precipitates, the result was similar to that obtained in the case of trypsin. Both antigen and precipitin were demonstrable in the supernatant fluid. It should be remembered, in this connection, that the leucocytes were derived from the same species of animal which yielded the immune serum, namely, the rabbit. The result of one such experiment is appended in tabular form.

TABLE II

SERUM 894 R. > H. S.	HORSE SERUM	PRECIPITATE			
		Leucocyte extract		Sodium chloride extract	
		+ 0.1 cc. horse serum	+ 0.5 cc. Serum 894	+ 0.1 cc. horse serum	+ 0.5 cc. Serum 894
cc.					
1	0.1	+	+	—	Slight
	0.01	+	+	—	Slight
	0.001	+	Slight	—	Slight

DISSOCIATION WITH CRYSTALLINE EGG ALBUMEN

The experiments in dissociation hitherto described have dealt with precipitates composed of horse serum and its antiserum. In order to complete this study, it seemed advisable to determine whether precipitates composed of a pure antigenic substance would yield similar results, and for this purpose precipitates composed of crystalline egg albumen and its antiserum were submitted to a similar study.

Experiment. To 2 cc. of Serum 883, rabbit versus crystalline egg albumen, are added in three separate tubes, respectively, 0.02 cc., 0.002 cc., and 0.0002 cc. of crystalline egg albumen. Control tests of the precipitates produced by one-half of these amounts in previous experiments (see p. 6) had shown that the first two combinations sensitized guinea pigs passively towards egg albumen, so that the antigen, injected three days later, induced immediate anaphylactic death; the precipitate of the third mixture, however, gave only a moderate degree of passive sensitization. In the present experiment, the resulting precipitates were washed, and were separately extracted with sodium carbonate in the incubator. After centrifugation, one-half of each tube was reserved for tests of the extract, which revealed the presence of precipitable egg albumen, while no precipitin could be demonstrated. The remainder was injected into three guinea pigs. After an interval of three days these pigs were given an injection of crystalline egg albumen intravenously. The results were as follows:

TABLE III

1 cc. of Serum 883, and graded amounts of crystalline egg albumen, as follows:

+ CRYST. EGG ALBUMEN	SUPERNATANT FLUID		CARBONATE EXTRACT OF PRECIPITATE INTO GUINEA PIG	SYMPTOMS ON INJECTION OF EGG ALBUMEN
	+ egg albumen	+ 883		
0.01	—	++	1	Moderate
0.001	—	+	2	Severe
0.0,001	—	—	3	None

Serum 883 sensitized guinea pigs in amounts of 0.05 cc. If 1 cc. was used as the sensitizing dose, desensitization was produced by a preliminary subcutaneous injection of 0.05 to 0.1 cc.

The results of this experiment are in harmony with those previously described. The extract contains antigen in precipitable form. Antibody is also present, but no longer possesses the function of a precipitin; it can, however, be demonstrated by passive sensitization.

Similar experiments, but less complete, were done with salt solution, and gave similar, but less striking results. Trypsin, and leucocytes, were not tried.

MECHANISM OF DISSOCIATION

The mechanism of dissociation offers certain problems of interest. Perhaps of the greatest significance, because of its apparent simplicity, is the form of dissociation which occurs in salt solution. Naturally, there is a tendency to explain this phenomenon in terms of the laws of mass action. Such a comparison, however, meets with so many initial difficulties that its application to the present problem becomes of doubtful value. According to the laws of mass action it is indeed true that dissociation of a precipitate occurs if the supernatant fluid is removed, and fresh fluid is added. But in this case the supernatant fluid in the first instance always contains both constituents of the reaction. It has been shown, however, that antigen and antibody do not coexist in the supernatant fluid, yet dissociation does occur. It is, however, also true that in inorganic chemistry a mixture of two reacting substances, of which one is in great excess, may leave only that one of them in the supernatant fluid, and that here dissociation of both constituents might be effective. So that, in this instance, the conditions appear more nearly analogous. A second difficulty is due to the fact that the dissociated factors differ from the original factors of the precipitation reaction, inasmuch as the dissociated antibody has lost its precipitating power. With the help of a variety of unsubstantiated auxiliary hypotheses, it would doubtless be possible to devise some sort of a plausible explanation reconciling these two types of phenomena. It seems best, however, to avoid this temptation, and to limit ourselves for the present to the search for facts. The increase of our knowledge of the

laws of reaction in immunology, coincident with a similar development in physical chemistry, may then very probably permit of a more satisfactory comparison of these two sets of phenomena.

THEORETICAL CONSIDERATIONS

From the above experiments, certain conclusions as to facts, in the first place, can be drawn. It is clear that the precipitate represents a complex of both factors, antigen and antibody, and that neither very largely destroys the other, if, indeed, any destruction at all occurs. The presence of both factors in demonstrable form in the extracts suggests various possible explanations. In the first place, the two substances may be separate in the extract, having lost their previous affinity. In the second place, they may be united. The latter assumption would seem to imply that they are in such proportions as constitutes the prozone, in which excess of antigen inhibits precipitation. This might be explained on the hypothesis of a redistribution of antibody in the precipitate. Such a process is hard to imagine; yet it is well known that such a redistribution of hemolysin or agglutinin may occur. Opposed to this view is the fact that the extracts produce passive sensitization, while the prozone fluid, in the case of horse serum, does not sensitize passively, on account of excess of antigen. Apparently, therefore, one must conclude that the two substances are present in proportions different from that of the prozone. If such be the case, and they are in combination, it is difficult to understand why the combination is present as a solution, instead of as a precipitate. Assuming, on the other hand, that the two factors are not in combination, there are difficulties again in explaining this condition. Passive sensitization proves that the antibody still retains its affinity for the antigen; how then, can it exist in solution therewith, without entering into combination? Thus it is impossible, with our present knowledge of the facts, to give any satisfactory explanation for the presence of antigen and antibody in the extracts.

The differences between the results herein recorded, and those of Chickering indicate that general laws are not at present determinable. It seems possible that the failure in his experiments to demonstrate precipitinogen may be analogous to the failure in the present experiments to demonstrate precipitin. It is well known that antigen may be so changed by heat, and other agencies, as to lose its precipitability, while it still retains its affinity for the precipitin. Thus, it might be necessary to test for the presence of antigen in extracts by the method of active sensitization, just as passive sensitization was used in the present series to demonstrate non-precipitating antibody.

CONCLUSIONS

Experimental data

1. Precipitates contain both antigen and antibody, as shown by the fact that they sensitize both actively and passively.

2. If precipitates are treated with salt solution in the incubator, the extracts are found to contain a small amount of antigen, but no antibody.

3. If precipitates are extracted with solutions of sodium carbonate, antigen is readily demonstrable in the extracts. Precipitin cannot be demonstrated, but antibody is demonstrable in large amounts, by the method of passive sensitization (sensitizin).

4. Extraction with trypsin and with leucocytes yields both precipitin and precipitinogen.

Theoretical considerations

Extraction of precipitates yields both components, but the procedure may deprive the antibody of its precipitating function. To what factor this change is to be ascribed, cannot be positively stated. It is possible that the antigen and antibody in the extract are not actually dissociated, but are in solution in combined form.

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STUDIES IN ANAPHYLAXIS

XVII. ON THE COEXISTENCE OF ANTIGEN AND ANTIBODY IN THE BODY

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THEORETICAL DISCUSSION

The starting point of the preceding studies was the observation that antigen and antibody had been found to coexist in reactive form in the body. Eisenberg's observation seemed to furnish a striking analogy to this phenomenon in the test-tube. With the changed interpretation of this observation, its value as an analogy disappears. Hence, it again becomes of interest to inquire whether the studies on equilibrium in precipitation have shed any further light on the problem of coexistence of antigen and antibody in the body.

If the preceding data be analyzed, it is evident in the first place that in the form of precipitate antigen and antibody can coexist as independently demonstrable entities, although in combination. Coexistence in such a state, namely, as precipitate, is, of course, hardly comparable with coexistence in the blood, or within the cells. It has been shown that in solution precipitin and precipitinogen cannot coexist without undergoing combination and precipitation. As far as one can reason from the test-tube to vital conditions, therefore, it is a valid assumption that these two factors cannot coexist in the living body in solution—that is to say, in the blood. Whether or not the cell can keep two substances of this sort apart, in spite of their high affinity is not, with the facts at present available, a question which admits of discussion.

On the other hand, the preceding papers have disclosed a set of conditions in the test-tube which seem to offer certain analogies with those previously described in the body. It has been definitely shown that antigen and antibody can coexist in the same fluid, provided that the antibody does not possess a precipitating function. Such is the case, for example, when precipitin has been heated to 70° . Here, however, we are dealing with an artificial condition which could not be duplicated in the living animal. The experiments in the equilibrium of the precipitation reaction, both in combination and in dissociation, however, present another phenomenon, which might conceivably be paralleled, or duplicated, in the animal body. The dissociation of precipitates in the presence of salt solution, yields a fluid containing both antigen and antibody. It does not seem unlikely that processes similar, if not identical, may produce similar results in the body. Again, under certain conditions the prozone presents an unprecipitated combination of antigen and antibody, in which both factors are demonstrable. Unquestionably, similar combinations might exist in the blood.

There is a gap, however, in the preceding comparison. Although the antibody of the blood, and that in the extracts and prozone fluid, have been demonstrated by the same method, namely, that of passive sensitization, the antibody in the cells is demonstrated by its anaphylactic response, its immediate reactivity with antigen. The further question arises, therefore, whether antibody in combination with antigen possess the capacity to react with fresh increments of antigen. This question is one which may be answered from the experiments of other observers. Bordet (1) Morgenroth (4), and Muir (5) have studied the behavior of hemolysin already anchored to red blood cells, in the presence of fresh red cells. There is no possibility of doubt but that the anchored hemolysin can and does sensitize the newly added cells. Landsteiner (2, 3) has found that agglutinin acts in the same manner. Thus, of these types of antibody it is clear that they can "fix" fresh antigen and react therewith, in spite of the fact that they are already in combination with antigen. The mechanism of this process is a matter of some

speculation. Those who accept Ehrlich's theory, naturally have an explanation which differs from that offered by Bordet. But concerning the facts themselves there is no dispute. If fixed antibody can react in the test-tube with fresh antigen, there seems to be no reason why the same mechanism may not be effective in the living body. Presumably the reactivity of fixed antibody would not be so high as that of free antibody, and, indeed, we find that this is exactly the case in the body.

CONCLUSIONS

1. It has been shown that antigen and antibody may coexist in the same fluids in the test-tube in reactive form.
2. It is known that fixed antibody may attack fresh antigen.
3. It is, therefore, quite in accordance with observations on immune reactions in the test-tube, to maintain that antigen and antibody may coexist in the blood and in the cells of the living animal, and that, even if in combination with antigen, the antibody is still capable of reacting with fresh antigen.

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COMPLEMENT FIXATION IN VARICELLA

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As one attack of varicella usually confers an active immunity for the balance of life it is probable that antibodies to the virus persist for long periods of time in the body fluids. Few persons possess a natural immunity to this disease as the majority of those escaping infection in childhood contract the disease later in life.

Clinical experience and experimental data indicate quite conclusively that the virus of this disease is present in the skin lesions as in variola; while the exact mode of transmission is unknown, the consensus of opinion is to the effect that the virus is inspired and in this manner reaches the body fluids and later the skin for which the virus apparently possesses a selective tissue affinity.

The diagnosis of varicella usually presents no difficulties on account of the mildness of the general reaction and the more or less typical appearance of the cutaneous lesions. Not infrequently however, the disease is confused with variola and syphilis; the lesions may resemble the vesicles of variola so closely that differentiation is difficult. The two diseases however, are distinct as clearly indicated by the fact that one who has recovered from varicella is susceptible to cowpox vaccination and that an attack of varicella does not protect against variola.

The object of this investigation was to determine if antibodies could be detected in the blood serum of persons actively infected with varicella and after recovery, by means of complement fixation tests using as antigens extracts of the contents of the vesicles and crusts of this disease. Further than this it was

considered of interest to study by means of complement fixation experiments, the possible relationship between varicella and variola, purely on the basis of the close similarity between the diseases in so far as route of infection, the mechanism of the production of lesions and the clinical similarity of the lesions, are concerned.

MATERIALS AND METHOD OF STUDY

Hemolytic system. The antishoop hemolytic system was employed. Complement was furnished by the pooled sera of two of more guinea-pigs and used in constant dose of 1 cc. of a 1:20 dilution (= 0.05 cc. undiluted serum). Antishoop hemolysin (rabbit) was titrated each time with each complement serum and a 2.5 per cent suspension of washed sheep cells (dose 1 cc.) and used in the antigen titrations and complement fixation tests in an amount equal to two hemolytic units. In this manner the hemolytic system was adjusted to each complement serum and corpuscle suspension and the double unit of hemolysin insured against non specific reactions in the antigen titrations and complement fixation tests.

Technic. In titrating the antigens increasing doses of each extract were incubated for an hour at 37° C. with a constant dose of 1 cc. of 1:20 dilution of the pooled complement sera when the corpuscles and two units of hemolysin were added; after a second period of incubation of an hour the results were read, the smallest dose of antigen causing slight inhibition of hemolysis being regarded as the anticomplementary dose. In the complement fixation tests one-third to one-half this dose was employed.

In the complement fixation tests inactivated serum, antigen and complement were incubated for an hour; corpuscles and two units of hemolysin were added and the tubes re-incubated for an hour or an hour and a half until the controls showed complete hemolysis, when the results were read.

In several experiments the complement fixation tests were conducted by primary incubations in the refrigerator (about

10° to 12° C.) for a period of six hours (McNeil) following which corpuscles and hemolysin were added and the tubes placed in an incubator at 37° C. for an hour or an hour and a half when the results were read.

As usual in complement fixation work serum, antigen, hemolytic, corpuscle etc., controls were employed in each test.

Antigens. Two varicella antigens were employed and in addition to these a salt solution extract of cowpox virus and a salt solution extract of variola crusts.

TABLE 1
Anticomplementary titration of antigens

DOSE	ANTIGENS			
	(1) NaCl extract vari- cella crusts	(2) NaCl extract vari- cella vesicle fluid	(3) NaCl extract cow- pox virus	(4) NaCl extract vari- ola crusts
cc.				
0.2	H	H	H	H
0.4	H	SIH	H	H
0.6	H	SIH	SIH	H
0.8	H	MIH	MIH	H
1.0	SIH	IH	MIH	SIH
1.2	MIH	IH	IH	MIH
Dose used	0.4 cc.	0.15 cc.	0.2 cc.	0.3 cc.

H = Complete hemolysis.

SIH = Slight (25 per cent) inhibition of hemolysis.

MIH = Marked (50 per cent) inhibition of hemolysis.

IH = Complete (100 per cent) inhibition of hemolysis.

No. 1. This antigen was prepared by collecting from a number of patients with varicella a quantity of dried crusts and extracting these with salt solution in the proportion of 4 cc. of solution to each 0.1 gram of dry crusts. The crusts were thoroughly ground with sterile powdered quartz, the salt solution plus 0.5 per cent phenol added and the whole shaken mechanically for twenty-four hours followed by incubation at 37°C. for seven days; this extract was then centrifuged at high speed, filtered through paper and titrated.

No. 2. This antigen was prepared by collecting the fluid from varicella vesicles into normal salt solution containing 0.5 per cent phenol and 0.2 per cent sodium citrate (to guard against the formation of a

coagulum). The amount of vesicle fluid could not be accurately measured but roughly estimated about two to four drops to each cubic centimeter of diluent. After standing several days this extract was filtered through paper and titrated.

No. 3. A 2 per cent salt solution extract of fresh cowpox virus. Fresh calf virus pulp was weighed, thoroughly ground with sterile quartz and mixed with sufficient normal salt solution containing 0.5 per cent suspension. After shaking for twenty-four hours the mixture was incubated at 37°C. for a week; centrifuged; filtered through paper and titrated.

No. 4. A salt solution of extract of dry variola crusts prepared in the same manner as antigen I (varicella) in the proportion of 0.6 gram of crust to 40 cc. of normal salt solution containing 0.5 per cent phenol.

Each serum was also tested for syphilis with a cholesterinized alcoholic extract of human heart. This extract was antigenic in 0.05 cc. of 1 : 20 dilution; 0.2 cc. (four antigenic units) was used as the dose as this amount was twelve times less than the anticomplementary unit.

Sera. All sera were inactivated at 56° C. for half an hour and used in constant dose of 0.2 cc.

SUMMARY OF RESULTS

The results of complement fixation reactions with sera from varicella patients are shown in tables 2 and 3 and may be summarized as follows:

1. Of 28 sera from patients who presented the first evidences of varicella in from four to forty-two days prior to the tests, 11 or 39 per cent reacted weakly positive with a salt solution antigen of varicella crusts.

2. Of 24 sera tested with a salt solution antigen of the contents of varicella vesicles, 7 or about 30 per cent reacted weakly positive.

3. Negative reactions with both varicella antigens were generally observed with the sera of adult persons who had varicella in childhood. In varicella the Wassermann reaction is negative in the absence of syphilis.

4. The sera of several syphilitics yielding strongly positive Wassermann reactions reacted negatively with the varicella antigens (no. 5, table 2; nos. 21 and 27, table 3).

TABLE 2
Complement fixation in varicella

NO.	NAME	AGE	VARICELLA DAY OF DISEASE	WASSER- MANN REACTION	ANTIGENS				VACCINATED
					(1) Vari- cella crusts	(2) Vari- cella vesicle fluid	(3) Cow- pox virus	(4) Variola crusts	
		<i>years</i>							
1	H. F.	5	10th	—	+	0	±	—	Yes
2	S. C.	7	16th	—	±	0	—	—	Yes
3	E. H.	4	17th	—	—	0	—	—	No
4	V. M. A.	7 wks.	4th	—	+	0	—	—	No
5	C. McG.	28	16th of syph- ilis not varicella	++++	—	—	±	0	Yes
6	M. R.	8	14th	—	±	±	—	—	Yes
7	M. R.	3	13th	—	—	—	—	—	Yes
8	N. C.	3	4th	—	±	±	—	—	No
9	A. G.	6	12th	—	+	+	—	—	Yes
10	E. F.	3	11th	—	+	0	±	—	Yes
11	J. R.	5	14th	—	—	—	—	—	No
12	M. G.	3	15th	—	+	+	—	—	No
13	E. P.	10	17th	—	—	—	—	—	Yes
14	E. V.	5	1 year	—	—	—	—	—	Yes
15	J. D.	$\frac{3}{4}$	10th	—	—	—	—	—	No
16	S. F.	$\frac{3}{4}$	12th	—	—	—	—	—	No
17	B. W.	$\frac{3}{4}$	12th	—	—	—	—	—	No
18	M. R.	40	7th	—	—	0	0	—	Never vac- cinated
19	H. B.	1	10th	—	+	+	±	—	Yes

— = Complete hemolysis (negative).

± = Less than 25 per cent inhibition of hemolysis (doubtful).

± = 25 per cent inhibition of hemolysis (weakly positive).

++ = 50 per cent inhibition of hemolysis (moderately positive).

+++ = 75 per cent inhibition of hemolysis (strongly positive).

++++ = 100 per cent inhibition of hemolysis (strongly positive).

5. In all instances the degree of complement absorption with the sera of varicella patients and varicella antigens was slight (reactions weakly or doubtfully positive).

TABLE 3

Complement fixation in varicella with primary incubation at 37° C. and 10° to 12° C.

NO.	NAME	AGE	VARICELLA DAY OF DISEASE	WASSER- MANN REACTION	REACTION AT 37° C.				REACTION AT 10° TO 12° C.			
					(1) Vari- cella crusts	(2) Vari- cella vesicle fluid	(3) Cow- pox virus	(4) Variola crusts	(1) Varicella crusts	(2) Vari- cella vesicle fluid	(3) Cow- pox virus	(4) Variola crusts
		yrs.										
20	P. B.	4	8th	—	—	—	—	—	—	—	—	—
21	L. C.	5	8th	++++	—	—	—	—	—	—	—	—
22	C. G.	10	10th	—	—	—	—	—	±	±	—	—
23	H. H.	6	10th	—	+	+	—	—	+	+	—	—
24	A. H.	4	4th	—	—	—	—	—	—	—	—	—
25	R. H.	3	42nd	—	—	—	—	—	—	—	—	—
26	T. M.	6	21st	—	++	++	—	—	++++	++++	—	—
27	S. S.	2½	2nd	++++	—	—	—	—	—	—	—	—
28	F. T.	3	19th	—	—	—	—	—	—	—	—	—
29	D. P.	5	10th	—	—	—	—	—	—	—	—	—
30	F. W.	6	11th	—	—	—	—	—	—	—	—	—
31	J. K.	29	23 yrs.	—	—	—	0	0	—	—	0	0
32	M. T.	32	27 yrs.	—	—	—	0	0	—	—	0	0
33	E. M.	26	20 yrs.	—	—	—	0	0	—	—	0	0
34	A. A.	34	30 yrs.	—	—	—	0	0	—	—	0	0
35	L. F.	23	20 yrs.	—	—	—	0	0	—	—	0	0
36	F. S.	27	22 yrs.	—	—	—	0	0	—	—	0	0

TABLE 4

Complement fixation reaction with cowpox immune sera and varicella antigens

ANIMAL	DAYS AFTER INOC - LATION WITH COW- POX VIRUS	REACTIONS WITH ACTIVE SERUMS (0.2 cc.)				REACTIONS WITH HEATED SERUMS (0.2 cc.)			
		Wasser- mann reac- tion	(2) Vari- cella vesicle fluid	(3) Cowpox virus	(4) Variola crusts	Wasser- mann reac- tion	(2) Vari- cella vesicle fluid	(3) Cowpox virus	(4) Variola crusts
Rabbit 60..	15	—	—	++++	++++	—	—	++++	++++
Rabbit 61..	15	—	—	++++	++	—	—	++++	++++
Rabbit 63..	12	—	—	+++	++	++	++	++++	++++
Rabbit 64..	12	—	—	++	++	+	+	++++	+++
Rabbit 64..	21	—	—	+++	++	++	++	++++	++++
Rabbit 66..	4	—	—	—	—	—	—	—	—
Rabbit 66..	14	—	—	++	±	—	—	+++	++
Calf (A)....	16	—	—	+	—	—	—	++	±
Calf (B)....	21	—	—	+++	+	—	—	+++	++

6. Cowpox and variola antibodies did not absorb complement with varicella antigens. The four weakly positive reactions observed with a salt solution extract of cowpox virus (calf) are to be ascribed to the presence of cowpox antibodies in the sera (table 2, nos. 1, 5, 10, and 19), similar results having been observed in the study of complement fixations in vaccinia and variola (1).

As shown in Table 4 the active sera of rabbits and calves vaccinated with cowpox virus yielded well marked positive reactions with antigens of cowpox and variola material but negatively with the antigen prepared of the contents of the vesicles of

TABLE 5
Complement fixation with the sera of smallpox and varicella antigens

NO.	AGE	SMALLPOX	DAY OF DISEASE	VARICELLA	WASSER-MANN REACTION	ANTIGENS		
						(2) Varicella vesicle fluid	(3) Cow-pox virus	(4) Variola crusts
1	50	Moderate	9th	Childhood	—	—	—	—
2	38	Very mild	33rd	Childhood	—	—	—	—
3	13	Very mild	31st	10 years ago	—	—	—	—
4	10	Mild	16th	6 years ago	—	—	+	+
5	21	Mild	17th	Childhood	—	—	++	+
6	11	Very mild	20th	10 yrs. ago	—	—	—	—
7	24	Mild	20th	Childhood	—	—	+	+
8	8	Mild	7th	4 years ago	—	—	++	++

varicella. Inactivated rabbit sera (heated at 56° C. for thirty minutes) reacted positively not only with varicella antigen but also with the cholesterinized alcoholic extract of heart (table 4, rabbits 63 and 64) constituting examples of non-specific complement fixation sometimes found with normal rabbit serum (2) and likely to be regarded as specific reactions.

As shown in table 5 negative reactions likewise occurred with the sera of eight persons suffering with a mild form of variola and an antigen of the fluid from varicella vesicles.

7. While these experiments have shown that an antibody in the nature of an amboceptor is present in the sera of persons

suffering with varicella which will absorb complement in the presence of an antigen prepared of the cutaneous lesions of this disease, yet the percentage of positive reactions and particularly the degree of complement absorption is small; while immunity principles are in all probability present in the body fluids of persons for years after an attack of varicella these could not be detected by the complement fixation tests in this study; all positive reactions were observed during or soon after an attack of the disease and at the time of probable highest concentration of antibodies. A more delicate technic would probably yield a higher percentage of positive reactions as is usual in all complement fixation tests with bacterial antigens, but in this study this was avoided in order to guard against the possibility of non-specific absorption of complement.

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COMPLEMENT FIXATION IN VACCINIA AND VARIOLA

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While clinical experience since the days of Jenner has conclusively proven that successful vaccination with cowpox virus or with the virus of smallpox itself results in establishing a high degree of active immunity against smallpox our knowledge of these protective antibodies is quite limited. Likewise clinical experience indicates quite conclusively that the antibody produced by inoculation with cowpox virus must be identical with or almost so with the antibody produced by smallpox virus, but the establishment of this relation between the two antibodies by means of immunological reactions has been less satisfactory, due probably to our ignorance of the specific micro-organisms of those diseases, our imperfect knowledge of the nature of the antibodies and the present limitations of immunological technic.

Attempts toward inducing passive immunization of animals against cowpox and smallpox by means of the injection of blood from vaccinated animals have been occasionally successful and frequently unsuccessful; likewise in a few experiments the virus of cowpox has been successfully inactivated or destroyed in vitro through contact with the blood serum of vaccinated animals. These experimental demonstrations have been generally unsatisfactory but on account of their crude nature have in no way militated against the overwhelming clinical experience indicating the intimate relationship between the antibodies of variola and vaccinia.

During the past three years I have had the opportunity of studying a number of cases of smallpox in the Philadelphia Hospital for Contagious Diseases and in Millville, New Jersey. All

of these persons exhibited a mild form of the disease in which the lesions were comparatively few, the systemic symptoms mild and recovery the rule. In the course of these studies complement fixation reactions were undertaken to throw more light upon the phenomenon of complement fixation in variola and vaccinia and in an effort to study the relation of the antibodies of variola and vaccinia by means of a series of cross complement fixation tests.

In 1906 Jobling (1) reported positive complement fixation reactions using as antigen a suspension of finely ground calf vaccine pulp and the sera of vaccinated calves. As far as I am aware this work constitutes the first investigation of its kind conducted in this field and indeed was in progress at the time of the original complement fixation work of Wassermann and his co-workers and Detre in syphilis. In 1909 Beintker (2) reported indifferent results in which he used as antigen a salt solution extract of calf lymph and the sera of vaccinated rabbits and calves as well as the sera of persons suffering with smallpox. In the same year Sugai (3) using as antigens salt solution extracts of the contents of smallpox pustules and calf lymph reported positive reactions with the sera of six smallpox patients and five persons who had been vaccinated with calf lymph. He found no evidence of agglutination in an extract of calf virus material by these sera. Dalm (4) reported positive results with the sera of ten persons suffering with smallpox and an antigen of calf lymph; he also used watery extracts of the liver and spleen of a two-year-old child who had succumbed to smallpox, but with negative or indifferent results; this investigator also found agglutination of calf lymph by the sera of smallpox patients to be weak or entirely absent. Kryloff (5) using a salt solution extract of variola matter reported positive reactions in variola and varioloid and considered the complement-fixation test as possessing diagnostic value. Aqueous and alcoholic extracts of variolous scabs and the liver and spleen of persons dead of variola as well as salt solution extracts of calf lymph yielded negative or weaker reactions. Bermbach (6) tested the sera of animals before and after vaccination and the sera of vaccinated and revaccinated persons, with a salt solution extract of lymph

and reported generally positive reactions. Xylander (7) tested thirty-one sera of persons before and after vaccination with an alcoholic extract of lymph, in an effort to study the specificity of the Wassermann reaction in syphilis. Of these the sera of eight reacted weakly positive with the lymph antigens and seven of these reacted in the same manner with the alcoholic extract of heart; after vaccination eighteen reacted weakly positive with the lymph antigen and these included the sera of the same seven persons who reacted weakly positive with the alcoholic heart antigen prior to vaccination. Teisser and Gastinell (8) using as antigen a salt solution extract of calf lymph reported positive reactions with the sera of thirty-nine persons suffering with variola, the reactions becoming positive about the tenth day of the disease and remaining so for at least thirty days; more prolonged observations were not made. Positive reactions were also reported with the sera of vaccinated rabbits, the reactions becoming positive about the seventh day after vaccination. Recently Klein (9) has also reported favorable results with the complement fixation test in variola especially with antigens prepared of the contents of the vesicles and pustules of variola and believe the test to possess practical diagnostic value. Kanschegg (10) has also reported positive reactions in variola with salt solution extracts of the contents of variolous lesions.

In my work antigens for the complement-fixation tests were prepared of the contents of vesicles and pustules and the scabs of vaccinia and vaccine pulp from calves and from the contents of vesicles and pustules and scabs of small-pox patients. With these antigens complement fixation reactions were conducted with homologous antigens and sera and the immunological relationship between vaccinia and variola studied by crossing the antigens and sera in complement-fixation experiments.

MATERIALS AND METHOD OF STUDY

Hemolytic system and technic. The antisheep hemolytic system was used throughout. Complement was furnished by the pooled sera of several guinea-pigs and used in doses of 1 cc. of a 1 : 20 dilution (= 0.05 cc. undiluted serum). Antisheep hemolysin

was titrated each time with this unit of complement and 1 cc. of a 2.5 per cent suspension of washed sheep cells and used in the antigen titrations and complement fixation tests in an amount equal to double the hemolytic unit.

In the antigen titrations, increasing doses of antigen with the unit of complement were incubated for an hour at 37°C. when two units of hemolysin and 1 cc. of the corpuscle suspension were added, mixed and re-incubated for an hour when the readings were made. That amount of antigen showing beginning inhibition of hemolysis was taken as the anticomplementary dose.

In the complement-fixation reactions serum, antigen and complement were mixed and incubated for an hour when two units of hemolysin and the corpuscles were added. After a second period of one hour incubation the readings were made.

In all tests the usual antigen, serum, hemolytic, etc., controls were employed.

Antigens. During the course of this study a number of antigens were prepared of salt solution and alcoholic extracts of vaccine virus¹ the contents of smallpox vesicles and pustules as well as of the dried scabs of cowpox and smallpox.

These antigens were prepared as follows:

No. 1. A 2 per cent salt solution extract of fresh calf virus pulp; shaken mechanically for twenty-four hours and filtered through paper; heated at 60°C. for 1 hour and preserved with 0.5 per cent phenol.

No. 2. A 5 per cent salt solution extract of fresh rabbit virus preserved with 0.5 per cent phenol; shaken mechanically for twenty-four hours; incubated at 37°C. for seven days; filtered through paper.

No. 3. A 4 per cent extract of fresh calf virus pulp in absolute alcohol; shaken mechanically for twenty-four hours and filtered.

No. 4. A glycerinated calf virus was centrifuged and the sediment washed twice with normal salt solution; a 2 per cent extract in absolute alcohol was prepared; shaken mechanically for twenty-four hours and incubated at 37°C. for one week; filtered through fat free paper.

¹ For several lots of vaccine pulp several vaccinated rabbits and the sera of vaccinated calves, I am indebted to Dr. F. G. Elgin of the Mulford Biological Laboratory to whom I wish to express deep appreciation for repeated acts of kindness.

No. 5. A glycerinated calf virus was centrifuged and the sediment washed twice with normal salt solution, one gram of sediment was mixed with 60 cc. pure acetone; shaken mechanically for twenty-four hours and incubated at 37°C. for one week; filtered through paper.

No. 6. Four cubic centimeters of calf virus were suspended in 40 cc. salt solution; shaken mechanically and filtered through paper to remove large particles and precipitated with an equal volume of absolute alcohol; the precipitate was dried over calcium chloride, weighed and ground with sufficient crystals of sodium chloride to make a 2 per cent suspension with the addition of distilled water.

No. 7. An emulsion of the contents of smallpox vesicles and pustules in salt solution preserved with 0.5 per cent phenol shaken mechanically for twenty-four hours and filtered through paper.

No. 8. A 2 per cent suspension of smallpox scabs dried and powdered, in normal salt solution plus 0.5 per cent phenol; shaken mechanically for twenty-four hours and incubated at 37°C. for a week; filtered through paper.

No. 9. A 1 per cent suspension of powdered smallpox scabs (Millville case) in normal salt solution plus 0.5 per cent phenol; shaken mechanically for twenty-four hours and incubated at 37°C. for one week; filtered through paper.

No. 10. A 2 per cent suspension of powdered cowpox scabs (human) in normal salt solution plus 0.5 per cent phenol shaken mechanically for twenty-four hours and incubated at 37°C. for a week, filtered through paper.

No. 11. A 4-per cent extract of powdered smallpox scabs in absolute alcohol; shaken mechanically for twenty-four hours and filtered through paper.

No. 12. A 4 per cent extract of powdered cowpox scabs (human) in absolute alcohol; shaken mechanically for twenty-four hours and filtered through paper.

For the Wassermann reaction an alcoholic extract of human heart re-enforced with cholesterin was used as antigen. The antigenic unit of this antigen as determined by titration with syphilitic sera was 0.05 cc. of a 1 : 20 dilution; the dose employed was 0.2 cc. of a 1 : 20 dilution. This extract was not antitytic in amounts under 2 cc. of a 1:10 dilution.

Each antigen was diluted as necessary with normal salt solution and titrated to determine its anticomplementary unit, i.e.

the smallest dose of antigen which in the presence of normal serum caused beginning inhibition of hemolysis. For the complement fixation tests the antigenic dose was taken arbitrarily as equal to one-fourth the anticomplementary unit. With this margin of safety false reactions due to the antilytic action of excessive doses of antigen were avoided. Larger doses of antigen as one-half the anticomplementary doses yielded stronger and higher percentages of reactions but the work reported herein was conducted with one-quarter the anticomplementary doses of each antigen.

These titrations were made each time just before the complement fixation tests and the following table shows the results of one such titration with each antigen and the dose employed in the complement fixation tests.

TABLE 1
Anticomplementary titration of antigens

DOSE	ANTIGENS AND DILUTIONS										
	1 1:10	2 1:10	3 1:10	4 1:10	5 1:10	7 1:10	8 1:10	9 1:10	10 1:10	11 1:10	12 1:10
cc.											
0.1	H	H	H	H	H	H	H	H	H	H	H
0.2	H	H	H	H	H	H	H	H	H	H	H
0.4	H	H	H	SIH	SIH	H	H	H	H	H	H
0.6	H	H	H	MIH	MIH	H	H	SIH	H	H	H
0.8	H	H	H	IH	IH	H	H	MIH	H	H	H
1.0	H	SIH	H	IH	IH	H	H	IH	H	H	H
2.0	H	MIH	SIH	SIH	SIH	H	SIH	IH	H	SIH	SIH
3.0	SIH	IH	IH	H	H	SIH	MIH	IH	SIH	MIH	MIH
Dose used, cc....	0.8	0.2	0.5	0.1	0.1	0.8	0.5	0.2	0.8	0.5	0.5

Antigen No. 6 was used in dose equivalent to 2 mg. of dried substance; this amount was equal to about one-fourth the anticomplementary dose of this preparation.

H = Complete hemolysis.

SIH = Slight inhibition of hemolysis (25 per cent).

MIH = Marked inhibition of hemolysis (50 to 75 per cent).

IH = Complete inhibition of hemolysis (100 per cent).

Sera. The following sera were employed:

- a. Seventeen from cases of smallpox.
- b. Fourteen from persons who had been vaccinated with cowpox virus.
- c. Two from children who had never been vaccinated.
- d. Seven from persons suffering with syphilis; vaccinated; never had smallpox.
- e. Five from vaccinated rabbits. These animals were inoculated in the skin of the back.
- f. Two from vaccinated calves. These animals were inoculated in the skin of the abdomen in the usual manner.
- g. One from normal calf.

All sera were inactivated by heating at 56°C. for half an hour; the rabbit sera were used in complement fixation tests both before and after inactivation.

RESULTS

I. Complement fixation in vaccinated animals

a. *With the sera of vaccinated rabbits.* These results are shown in tables 2 and 3. These animals were heavily infected and succumbed in from three to four weeks after inoculation.

Rabbits 48 and 53 were selected from a group of normal animals because preliminary complement fixation tests with these antigens showed that their sera in both active and inactivated state did not yield non-specific reactions. These precautions should always be taken as otherwise false conclusions may be drawn on the basis of non-specific reactions as recently emphasized by my associates and myself in a series of studies on this subject of non-specific complement fixation by normal rabbit serum (11 and 12).

Rabbits 43, 44, 45 were inoculated for me by Dr. Elgin before preliminary complement fixation tests were made; the sera of two of these animals nos. 43 and 45 were inactivated and yielded slight degrees of non-specific complement fixation with the cholesterolized alcoholic extract of human heart; this tendency of

TABLE 2
Complement fixation in vaccinia with the sera of vaccinated rabbits

No.	History	ACTIVE SERUM (0.2 cc.)				INACTIVATED SERUM (0.2 cc.)			
		Wasser- mann reac- tion	Antigen 1 NaCl extract cowpox virus	Antigen 7 NaCl extract variola	Serum control	Wasser- mann reac- tion	Antigen 1 NaCl extract cowpox virus	Antigen 7 NaCl extract variola	Serum control
48	Before vaccination.....	—	—	—	—	—	—	—	—
48	4 days after vaccination.....	—	—	—	—	—	—	—	—
48	7 days after vaccination.....	—	—	—	—	—	+	+	—
48	11 days after vaccination.....	—	++	+	—	—	++	+	—
48	24 days after vaccination.....	—	++	++	—	—	++	++	—
53	Before vaccination.....	—	—	—	—	—	—	—	—
53	4 days after vaccination.....	—	—	—	—	—	—	—	—
53	7 days after vaccination.....	—	—	—	—	—	++	++	—
53	11 days after vaccination.....	—	++	++	—	—	++	++	—
53	24 days after vaccination.....	—	++	++	—	—	++	++	—
43	12 days after vaccination.....	—	++	++	—	+	++	++	—
43	18 days after vaccination.....	—	++	++	—	+	++	++	—
43	21 days after vaccination.....	—	++	++	—	+	++	++	—
44	11 days after vaccination.....	—	—	—	—	—	++	++	—
44	17 days after vaccination.....	—	++	++	—	—	++	++	—
44	20 days after vaccination.....	—	++	++	—	—	++	++	—
45	88 days after vaccination.....	—	++	++	—	+	++	++	—

— = Complete hemolysis (negative)
 + = 25 per cent inhibition of hemolysis (weekly positive).
 ++ = 50 per cent inhibition of hemolysis (moderately positive).
 +++ = 75 per cent inhibition of hemolysis (strongly positive).
 ++++ = 100 per cent inhibition of hemolysis (strongly positive).

TABLE 3
Complement fixation in vaccinia with the sera of vaccinated rabbits

NO.	HISTORY	DOSE SERUM	ACTIVE SERUM				INACTIVATED SERUM			
			(1) NaCl extract cowpox virus	(7) NaCl extract variola	(4) Alcoholic extract cowpox virus	Wasser- mann reac- tion	(1) NaCl extract cowpox virus	(7) NaCl extract variola	(4) Alcoholic extract cowpox virus	Wasser- mann reac- tion
48	Vaccinated 15 days...	cc.								
		0.01	++	-	0	-	0	0	0	0
		0.05	++	-	0	-	0	0	0	0
		0.1	++	++	0	-	0	0	0	0
		0.2	++	++	0	-	0	0	0	0
48	Vaccinated 25 days...	0.2	-	-	-	-	0	0	0	0
		Serum control								
		0.01	++	-	-	-	++	+	-	-
		0.05	++	+	-	-	++	+	-	-
		0.1	++	++	-	-	++	+	-	-
53	Vaccinated 15 days...	0.2	++	++	+	-	++	++	+	-
		0.2	-	-	-	-	-	-	-	-
		Serum control								
		0.01	-	-	0	-	0	0	0	0
		0.05	-	-	0	-	0	0	0	0
53	Vaccinated 25 days...	0.1	++	-	0	-	0	0	0	0
		0.2	++	+	0	-	0	0	0	0
		0.2	-	-	0	-	-	0	0	0
		Serum control								
		0.01	-	-	-	-	-	-	-	-
53	Vaccinated 25 days...	0.05	-	-	-	-	+	+	+	-
		0.1	++	+	-	-	++	++	+	-
		0.2	++	++	-	-	++	++	+	-
		0.2	-	-	-	-	+	+	+	-
		Serum control					-	-	-	-

heated serum no doubt influenced the reactions with the antigens of cowpox virus and variolous material.

As shown in table 2 antibodies were found in the sera of the inoculated rabbits about the eighth to the eleventh day after inoculation as determined by the complement fixation tests with active serum. With inactivated serum (heated at 56°C. for half an hour) the reactions were more delicate and positive reactions were observed on the seventh day after inoculation.

As shown in this table the complement fixation tests showed quite definitely that the sera of these inoculated rabbits contained a complement absorbing antibody presumably in the nature of an amboceptor and that the antibody produced by inoculation with cowpox virus while absorbing complement in best degree with the homologous antigen, also showed a close biological relationship to variola by reason of complement absorption with variola antigens.

The alcoholic extract of cowpox virus pulp proved of low antigenic sensitiveness which observation is in keeping with the general finding that alcohol does not serve well in extracting antigenic principles from bacteria and protozoa.

b. With the sera of vaccinated calves. The results of two complement fixation tests with the sera of two calves are shown in tables 4 and 5.

TABLE 4
Serum from a vaccinated calf (seventh day)

DOSE OF SERUM	ANTIGENS				
	(1) NaCl extract cowpox virus	(3) Alcoholic ex- tract cowpox virus	(6) Cowpox virus	(7) NaCl extract variola	(11) Alcoholic extract variola
cc.					
0.005	—	—	—	—	—
0.01	—	—	—	—	—
0.05	—	—	—	—	—
0.1	—	—	—	—	—
0.2	—	—	—	—	—
0.2	—	—	—	—	—
Serum control	—	—	—	—	—

TABLE 5
Serum from vaccinated calf (sixteenth day)

DOSE	ANTIGENS				
	(1) NaCl extract calf virus	(3) Alcoholic ex- tract calf virus	(6) Calf virus	(7) NaCl extract variola	(11) Alcoholic extract variola
cc.					
0.005	—	—	—	—	—
0.01	+	—	—	—	—
0.05	++	—	±	+	—
0.1	++	+	+	++	+
0.2	++++	+	++	+++	+
0.2					
Serum control	—	—	—	—	—

As shown in table 4 antibodies were not demonstrated on the seventh day after inoculation; as shown in table 5 antibodies were present in the serum of a calf inoculated sixteen days previously. As observed with the sera of inoculated rabbits, the cowpox antibodies absorbed complement better with the cowpox antigens than with the variola antigen, likewise alcoholic extracts of cowpox and variola viruses proved poor in antigenic sensitiveness.

As shown in table 6 normal calf serum does not show the tendency toward non-specific reactions as exhibited by normal rabbit sera. The sera of ten other normal calves yielded similar negative results with these antigens.

TABLE 6
Serum from a normal calf

DOSE SERUM	ANTIGENS				
	(1) NaCl extract cowpox virus	(2) Alcoholic ex- tract cowpox virus	(6) Cowpox virus	(7) NaCl extract variola	(11) Alcoholic extract variola
cc.					
0.005	—	—	—	—	—
0.01	—	—	—	—	—
0.05	—	—	—	—	—
0.1	—	—	—	—	—
0.2	—	—	—	—	—
0.2					
Serum control	—	—	—	—	—

II. Complement fixation in human vaccinia

The results of a number of complement fixation reactions with the sera of persons at varying intervals after successful vaccination with cowpox virus are shown in tables 7 to 11 inclusive.

Of 13 persons vaccinated with cowpox virus from seven days to ten years previously and whose sera yielded negative Wassermann reactions, positive reactions with salt solution antigens of cowpox virus were observed with four or 22 per cent. The sera of one of these four persons yielding positive reactions with cowpox virus also reacted positively with salt solution antigen of variolous material.

TABLE 7

Serum from person successfully vaccinated one week previously

SERUM	ANTIGENS			
	(1) NaCl extract cowpox virus	(3) NaCl extract smallpox scabs	(10) NaCl extract cowpox scabs	Wassermann reaction
cc.				
0.01	—	—	—	—
0.05	—	—	+	—
0.1	+	+	++	—
0.1				
Serum control	—	—	—	—

TABLE 8

Serum from a child vaccinated eight days previously

DOSE SERUM	ANTIGENS					
	(1) NaCl ex- tract cow- pox virus	(3) Alcoholic extract cowpox virus	(6) Cowpox virus	(7) NaCl extract variola	(11) Alcoholic extract variola	Wasser- mann reaction
cc.						
0.005	—	—	—	—	—	—
0.01	—	—	—	—	—	—
0.05	—	—	—	—	—	—
0.1	—	—	—	—	—	—
0.2	—	—	—	—	—	—
0.2						
Serum control	—	—	—	—	—	—

TABLE 9

Serum from person successfully vaccinated three weeks previously

SERUM	ANTIGEN			
	(1) NaCl extract cowpox virus	(7) NaCl extract smallpox scabs	(10) NaCl extract cowpox virus	Wassermann reaction
cc.				
0.01	—	—	—	—
0.05	—	—	—	—
0.1	—	—	—	—
0.1	—	—	—	—
Serum control	—	—	—	—

TABLE 10

Serum from person successfully vaccinated three weeks previously

SERUM	ANTIGENS			
	(1) NaCl extract cowpox virus	(7) NaCl extract smallpox scabs	(10) NaCl extract cowpox scabs	Wassermann reaction
cc.				
0.01	—	—	—	—
0.05	—	—	+	—
0.1	—	—	+	—
0.1	—	—	+	—
Serum control	—	—	—	—

The persons whose sera yielded the positive reactions were vaccinated 8, 21, 21 and 14 days previously.

As shown in table 11 the sera of seven vaccinated persons yielded positive Wassermann reactions; six of these (nos. 9, 10, 12, 13, 15 and 16) were untreated cases of syphilis; no. 11 was undergoing treatment at the time of vaccination and the complement fixation tests. Four persons (nos. 9, 10, 11 and 13) had been vaccinated in early childhood; the present vaccinations were successful but all were mild "takes;" nos. 14, 15 and 16 did not take at this time.

It will be noted that the alcoholic extract of cowpox virus yielded positive results with the sera of five of these persons and likewise a higher percentage of positive reactions resulted with

TABLE 11
Complement fixation after cowpox vaccination

NO.	NAME	AGE	VACCINATION	WASSER- MANN REACTION	ANTIGENS			
					(1) NaCl extract cowpox virus	(2) NaCl extract lymph	(8) NaCl extract variola scabs	(4) Al- coholic extract cowpox virus
		<i>years</i>						
1	O. M.	65	6 months	—	—	—	—	—
2	G. S.	41	18 days	—	—	—	—	—
3	T. T.	5	9 days	—	—	—	—	—
4	W. H.	49	14 days	—	+	+	—	—
5	J. M.	46	9 days	—	—	—	—	—
6	T. M.	44	10 weeks	—	—	—	—	—
7	T. S.	20	10 days	—	—	—	—	—
8	W. N.	33	10 years	—	—	—	—	—
9	S. B.	30	7 days	++++	++	++	++	++
10	J. W.	26	6 days	++++	+	+	—	+
11	J. R.	29	12 days	=	—	—	—	—
12	J. A.	32		++++	++	++	—	+
13	J. M.	18	14 days	++	—	—	—	—
14	J. W.	50	10 years	—	—	—	—	—
15	J. W.	27	4 years	++	+	—	—	=
16	J. F.	28	3 years	++++	=	=	—	++

the salt solution extracts of cowpox virus. I am inclined to regard these reactions particularly those resulting with the alcoholic extract of cowpox virus, as due to the presence of the syphilis "reagin" and its faculty for absorbing complement with lipid substances.

As shown in tables 12 and 13 the sera of persons never vaccinated with cowpox virus reacted negatively with all antigens.

According to the results observed the sera of but a small proportion of persons vaccinated with cowpox virus contained sufficient complement absorbing antibodies to yield positive reactions with the antigens used in this study.

The most of the positive reactions that were observed were but weakly positive and occurred among persons recently vaccinated. Ten additional tests not listed in the tables were conducted with the sera of adult persons who were successfully vaccinated in childhood and who were known to be immune at

TABLE 12

Serum of a child who had never been vaccinated

DOSE SERUM	ANTIGENS					
	(1) NaCl ex- tract cow- pox virus	(3) Alcoholic extract cowpox virus	(6) Cowpox virus	(7) NaCl extract variola	(11) Alcoholic extract variola	Wasser- mann reaction
cc.						
0.005	—	—	—	—	—	—
0.001	—	—	—	—	—	—
0.05	—	—	—	—	—	—
0.1	—	—	—	—	—	—
0.2	—	—	—	—	—	—
0.2	—	—	—	—	—	—
Serum control	—	—	—	—	—	—

TABLE 13

Serum of a child who had never been vaccinated

DOSE SERUM	ANTIGENS				
	(1) NaCl extract cowpox virus	(3) Alcoholic ex- tract cowpox virus	(5) Cowpox virus	(7) Alcoholic ex- tract variola	(11) Alcoholic ex- tract variola
cc.					
0.005	—	—	—	—	—
0.01	—	—	—	—	—
0.05	—	—	—	—	—
0.1	—	—	—	—	—
0.2	—	—	—	—	—
0.2	—	—	—	—	—
Serum control	—	—	—	—	—

the time of these tests by reason of the development of an immediate or "immunity reaction" upon inoculation with cowpox virus, an all yielded negative reactions with all antigens. Since antibodies were present and as these are probably in the nature of amboceptors it is probable that the complement fixation tests were not sufficiently delicate for their detection; that the antibody of cowpox will absorb complement with an extract of virus was shown by Jobling with the sera of vaccinated calves and confirmed by others and myself with the sera of vaccinated rabbits and calves as shown earlier in this paper.

As observed with the sera of vaccinated rabbits, the cowpox antibody absorbs complement best with its homologous antigen; of the four human sera absorbing complement with cowpox antigen one reacted in a similar manner with a variolous antigen. This relationship between the antibodies of vaccinia and variola was further shown in the complement fixation tests with the sera of persons with smallpox.

III. Complement fixation in variola

During the past three years I have had the opportunity of applying complement fixation tests with the sera of 17 persons during the active stages of smallpox.

TABLE 14

Serum from person with smallpox; eighteenth day of the disease; very mild infection

SERUM	ANTIGENS			
	(1) NaCl extract cowpox virus	(7) NaCl extract smallpox scabs	(10) NaCl extract cowpox scabs	Wassermann reaction
cc.				
0.01	—	—	—	—
0.05	—	—	—	—
0.1	—	+	+	—
0.1	—	—	—	—
Serum control				

TABLE 15

Serum of a smallpox patient; twenty-first day of disease; exceedingly mild case presenting few lesions and practically no systematic symptoms

DOSE SERUM	ANTIGENS					
	(1) NaCl ex- tract cow- pox virus	(3) Alcoholic extract cowpox virus	(6) Cowpox virus	(7) NaCl extract variola	(11) Alcoholic extract variola	Wassermann reaction
cc.						
0.005	—	—	—	—	—	—
0.01	—	—	—	—	—	—
0.05	—	—	—	—	—	—
0.1	+	—	—	+	—	—
0.2	++	—	—	++	—	—
0.2	—	—	—	—	—	—
Serum control						

TABLE 16

Serum of a patient with smallpox on nineteenth day of disease (fifteenth day after eruption); moderately severe infection; never vaccinated

DOSE SERUM	ANTIGENS					
	(1) NaCl extract cowpox virus	(3) Alcoholic extract cowpox virus	(6) Cowpox virus	(7) NaCl extract variola	(11) Alcoholic extract variola	Wasser- mann reaction
cc.						
0.005	—	—	—	—	—	—
0.01	—	—	—	—	—	—
0.05	—	—	—	—	—	—
0.1	+	—	—	+	—	—
0.2	++	—	—	++	—	—
0.2	—	—	—	—	—	—
Serum control						

TABLE 17

Serum of a patient who was exposed to smallpox for five days; vaccinated and vaccination was just beginning to "take" when patient developed mild symptoms of smallpox

DOSE SERUM	ANTIGENS					
	(1) NaCl extract cowpox virus	(3) Alcoholic extract cowpox virus	(6) Cowpox virus	(7) NaCl extract variola	(11) Alcoholic extract variola	Wasser- mann reaction
cc.						
0.005	—	—	—	—	—	—
0.01	—	—	—	—	—	—
0.05	—	—	—	—	—	—
0.1	+	—	—	+	—	—
0.2	++	—	—	++	—	—
0.2	—	—	—	—	—	—
Serum control						

The sera of nine of these persons were used in graded doses in the complement fixation tests in order to determine in this manner the relative degrees of complement absorption with cowpox and smallpox antigens. The results of these tests are shown in tables 14 to 22 inclusive. Table 23 shows a series of reactions with a constant dose of 0.2 cc. of serum with different antigens.

All of the cases were mild infections: several were exceedingly mild showing but few lesions and practically no systematic mani-

TABLE 18

Serum from person with smallpox; tenth day of disease; very mild infection

DOSE SERUM	ANTIGENS			
	(1) NaCl extract cowpox virus	(7) NaCl extract smallpox scabs	(10) NaCl extract cowpox virus	Wassermann reaction
cc.				
0.01	—	—	—	—
0.05	—	—	—	—
0.1	—	—	—	—
0.1	—	—	—	—
Serum control				

TABLE 19

Serum from person with smallpox; twelfth day of disease; very mild infection

DOSE SERUM	ANTIGENS			
	(1) NaCl extract cowpox virus	(7) NaCl extract smallpox scabs	(10) NaCl extract cowpox scabs	Wassermann reaction
cc.				
0.01	—	—	—	—
0.05	—	—	—	—
0.1	—	—	—	—
0.1	—	—	—	—
Serum control				

TABLE 20

Serum from patient with smallpox on the nineteenth day of illness and seventeenth day of eruption, mild infection; practically no symptoms

DOSE SERUM	ANTIGENS					
	(1) NaCl ex- tract cow- pox virus	(3) Alcoholic extract cowpox virus	(6) Cowpox virus	(7) NaCl extract variola	(11) Alcoholic extract variola	Wasser- mann reaction
cc.						
0.005	—	—	—	—	—	—
0.01	—	—	—	—	—	—
0.05	—	—	—	—	—	—
0.1	+	+	—	+	—	—
0.2	++	+	—	++	—	—
0.2	—	—	—	—	—	—
Serum control						

TABLE 21

Serum from patient with smallpox on the twenty-first day of the disease and seven-teenth day of eruption; mild infection; never vaccinated

DOSE SERUM	ANTIGENS					
	(1) NaCl ex- tract cow- pox virus	(3) Alcoholic extract cowpox virus	(6) Cowpox virus	(7) NaCl extract variola	(11) Alcoholic extract variola	Wasser- mann reaction
cc.						
0.005	—	—	—	—	—	—
0.01	—	—	—	—	—	—
0.05	—	—	—	—	—	—
0.1	—	—	—	—	—	—
0.2	—	—	—	—	—	—
0.2	—	—	—	—	—	—
Serum control						

TABLE 22

Serum from person that had smallpox thirty-nine years ago

DOSE SERUM	ANTIGENS					
	(1) NaCl ex- tract cow- pox virus	(3) Alcoholic extract cowpox virus	(6) Cowpox virus	(7) NaCl extract variola	(11) Alcoholic extract variola	Wasser- mann reaction
cc.						
0.005	—	—	—	—	—	—
0.01	—	—	—	—	—	—
0.05	—	—	—	—	—	—
0.1	—	—	—	—	—	—
0.2	—	—	—	—	—	—
0.2	—	—	—	—	—	—
Serum control						

festations. I am inclined to believe that the percentage of positive reactions and the degree of complement absorption would have been higher with the sera of persons with the severer type of smallpox.

The results of these examinations are summarized in table 24.

An examination of this table shows:

1. The sera of all patients reacted negatively in the Wassermann reaction.

TABLE 23

Complement fixation in variola

NO.	NAME	HISTORY OF VARIOLA	WASSERMANN REACTION	ANTIGENS		VACCINATION
				NaCl extract variola	NaCl extract cowpox virus	
1	A. G.	9th day; very mild	—	—	—	30 years ago
2	H. D.	33rd day; very mild	—	—	—	Never vaccinated
3	B. D.	31st day; very mild	—	—	—	Never vaccinated
4	H. D. D.	16th day; moderate	—	++	+	Never vaccinated
5	J. C.	17th day; mild	—	++	+	Never vaccinated
6	M. H.	20th day; very mild	—	—	—	Never vaccinated
7	J. D.	20th day; mild	—	++	+	Never vaccinated
8	R. G.	7th day; mild	—	++	+	Vaccinated 24 days ago

TABLE 24

Summary of complement fixation tests in variola (serum inactivated, dose 0.1 cc. to 0.2 cc.)

NO.	TABLE	VARIOLA	WASSER- MANN REACTION	ANTIGENS			
				NaCl ex- tract cow- pox virus	Alcoholic extract cowpox virus	NaCl extract variola	NaCl extract variola
1	14	Very mild	—	+	0	+	0
2	15	Very mild	—	++	—	++	—
3	16	Moderately severe	—	++	—	++	—
4	17	Mild	—	++	—	+++	—
5	18	Very mild	—	—	0	—	0
6	19	Very mild	—	—	0	—	0
7	29	Mild	—	++	+	++	—
8	21	Mild	—	—	—	—	—
9	22	39 years ago	—	—	—	—	—
10	23	Very mild	—	—	0	—	0
11	23	Very mild	—	—	0	—	0
12	23	Very mild	—	—	0	—	0
13	23	Moderate	—	+	0	++	0
14	23	Mild	—	+	0	++	0
15	23	Very mild	—	—	0	—	0
16	23	Mild	—	+	0	++	0
17	23	Mild	—	+	0	++	0

2. Of the 17 sera, 9 or about 60 per cent reacted positively with one of the salt solution extracts of variolous material (contents of lesions or extracts of scabs).

3. All sera reacting positively with the salt solution extracts of variolous material also reacted positively with a salt solution extracts of cowpox virus prepared of material from inoculated calves.

4. The degree of complement absorption was usually slightly greater with the variolous than with the cowpox antigens.

5. Alcoholic extracts of variolous and cowpox materials yielded uniformly negative results.

6. These reactions demonstrate the close biological relationship of the viruses of variola and vaccinia.

7. While this series of reactions was too small to determine the possible value of complement fixation tests in the diagnosis of variola it is probable that such tests may prove of value with a salt solution extract of variolous material particularly the contents of vesicles or a salt solution extract of fresh cowpox virus (calf or rabbit) as antigens. The sera from persons with severer infections are more likely to yield positive reactions than the sera from persons with mild and atypical infections.

CONCLUSIONS

1. The sera of rabbits inoculated with cowpox virus yielded positive complement fixation reactions with salt solution antigens of cowpox and smallpox viruses in seven to eight days after vaccination.

2. The antibody of cowpox virus in the sera of vaccinated animals showed a distinct and close biological relationship to the antigen of variola in complement fixation experiments.

3. Of 13 persons vaccinated with cowpox virus from seven days to ten years previously and whose sera yielded negative Wassermann reactions positive reactions with salt solution antigens of cowpox virus were observed with 4 or 22 per cent. The sera of one of these four persons yielding positive reactions (vaccinated 8, 21, 21 and 24 days previously) with cowpox virus,

reacted positively with a salt solution antigen of variolous material. The sera of unvaccinated persons reacted negatively with all antigens.

4. Of 17 persons suffering with mild smallpox, the sera of 9 or about 60 per cent yielded positive complement fixation reactions with salt solution antigens of variolous and cowpox viruses. While the degree of complement absorption was relatively weak in all instances the reactions were generally stronger with the variolous antigens than with the cowpox antigens.

5. Alcoholic extracts of variolous and cowpox viruses possessed little or no antigenic sensitiveness.

6. These complement fixation reactions have demonstrated the close biological relationship between the antibodies of vaccinia and variola; it is probable that complement fixation reactions with salt solution antigens of the contents of smallpox lesions or fresh cowpox virus will prove of some value in the diagnosis of smallpox.

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THE FATE OF VARIOUS ANTIBODIES IN THE PRECIPITIN REACTION

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Gay and Chickering (1) have recently shown that the antibodies in antipneumococcus serum from the horse which protect animals from infection with the pneumococcus, may be completely removed from the serum by specific precipitation produced by adding extracts of pneumococcus to the serum. Such precipitates laden with the antibodies will preserve animals from infection as well as the original serum from which they are derived and may be greatly concentrated. These antibody laden precipitates, moreover, contain only a fraction of the protein of the original serum at times only one-fiftieth or one-sixtieth of the amount. Although satisfactory protection and no particular harm followed the use of these protective precipitates in their original suspension, even when administered intravenously in animals, attempts were made to simplify the preparation by dissolving it. These attempts, which at first were not very successful, have since led to more satisfactory results in the hands of Chickering (2), who has investigated more closely the conditions under which the precipitate may best be produced for the purpose of bringing down all the antibodies, and has in particular shown that the protective bodies may be extracted from the precipitate by means of a dilute sodium carbonate solution at 42°C. This procedure still further reduces the protein content of the protective derivative and indicates that the protective antibodies are mechanically adsorbed by the specific precipitate.

The practical value of these observations is obvious and has

already been pointed out. The advantages that seem to attend the intravenous injection of large amounts of the strictly specific serum in two of the bacteriologically separate types of pneumonia would be greatly enhanced by the concentration of the immune principles in the manner we have described; certain of the disadvantages of the current method depending on the infusion of large amounts of a foreign protein will certainly be avoided. A further possible advantage lies in the fact that purified antibodies prepared in this way in reality have the advantage of a sensitized vaccine, that is they contain both antigen (from the bacterial extract added to produce the precipitate) and antibody, which in experimental animals rapidly gives rise to some degree at least of active as well as of passive immunity.

The observations recorded for the pneumococcus antiserum suggested two profitable lines of inquiry. First, an investigation of other antisera and antitoxins of recognized therapeutic value in reference to their possible concentration by similar methods of specific precipitation; and, secondly, the study of the properties of such purified antibodies as might be collected in this manner. This communication deals with the first series of data that have been collected along these lines.

An enormous literature represents the work that has been done on the theoretic and practical significance of specific precipitin reactions since the discovery of Kraus in 1897. This literature deals largely with the specificity, the forensic employment, and the physico-chemical conditions under which these reactions occur. Another considerable series of articles deals with the relation of precipitation to the reaction of alexin (complement) fixation or Bordet Gengou phenomenon. As one of us was first to point out (3) washed serum precipitates, at least under certain conditions, will fix alexin and indeed constitute, or better, contain the alexin fixing complex produced in a serum when mixed with its antiserum. More recent experiments (4) led us to believe that it is not the precipitate *per se* but rather the adherent antigen-antibody complex that fixes the alexin.

A not inconsiderable literature, which has in most part been referred to in our first article on pneumococcus precipitates,

indicates that in certain combinations other antibodies may be brought down in the precipitin reaction. The references to these antibody absorptions by specific precipitates, has been to a large extent incidental and at all events no systematic study of the behavior of the various types of antibodies in the presence of precipitate formation has been made. It will simplify the presentation of our own data and their relation to already determined facts if we consider certain of these type antibodies in turn in respect to their fate when they are present in a precipitate forming mixture.

ANTITOXINS

The most significant and best corroborated results bearing on the behavior of antibodies in the precipitin reaction are those derived from studies of antitoxins. Kraus and Eisenberg (5) endeavored to produce antagonistic substances ("anti-antibodies") to immune bodies derived from the horse, by immunizing rabbits either with normal horse serum or with antityphoid horse serum. The addition of tetanus antitoxin from the horse produced a precipitate in the immune rabbit serum but led to no diminution in the antitoxin content in the supernatant fluid. Similar results were obtained with the combination of rabbit antigoat serum and diphtheria antitoxin from the goat. The subsequent work of Dehne and Hamburger (6) showed that this failure to separate out antitoxin was due to the relative dosage of the reacting substances employed by Kraus and Eisenberg who used an excess of precipitinogen (horse and goat serum) which, as was later discovered, inhibits the maximum formation of precipitate. Dehne and Hamburger by diluting the antitoxin 1-500 produced the maximum precipitate which was found to contain all the antitoxin. This tetanus antitoxin could then be liberated from the precipitate by solution in an additional amount of antigen (horse serum). It is interesting to note that the whole undissolved precipitate does not protect and in this respect these results differ from ours with the pneumococcus protective precipitate. Dehne and Hamburger also noted that similar results may be produced in vivo for they found that the introduction of

tetanus toxin from the horse into rabbits immunized against horse serum leads to a rapid disappearance of the antitoxin with the precipitinogen. These experiments were fully corroborated by Kraus and Pribram (7), Hamburger (8), and von Eisler and Tsuru (9) in so far as tetanus antitoxin is concerned. Similar conditions were shown to exist for diphtheria antitoxin by Weill Hallé and Lemaire (10) and by Atkinson and Banzhaff. (11).

These observations, while of extreme theoretic interest, have led to no practical method of concentrating antitoxin owing to the extreme dilution of the antitoxin required to produce the maximum precipitate. In other words an excessive amount of antihorse serum (precipitin) would be required to precipitate any considerable amount of antitoxin (precipitinogen). It is precisely because our method of concentrating pneumococcus protective bodies is the reverse of this reaction that it is of potential practical value; the antibodies to be collected are in the precipitin serum, which is the reagent used in excess, instead of the precipitinogenic serum which is used in far smaller amounts. It seemed to us important to determine at once whether it would be possible to concentrate diphtheria antitoxin by producing precipitins as well as antitoxins in the immunized animal, in other words, by immunizing against *B. diphtheriae* as well as against its toxins.

Repeated attempts have been made to produce precipitins for the diphtheria bacillus by injections of increasing amounts of bacterial extracts, of dead, and later of living cultures of *B. diphtheriae* in rabbits. They have given entirely negative results. Through the courtesy and coöperation of Dr. Foster of the Cutter Analytic Laboratory, it has been possible to attempt a similar procedure in two horses that were already producers of diphtheria antitoxin. These two animals (nos. 103 and 107) were given in all 13½ cultures of an atoxic strain of *B. diphtheriae* on Loeffler's blood serum in seven spaced intravenous injections over one month. When their sera were tested a week after the last injection in doses of 1 cc., they gave no precipitin reaction with various doses of a clear extract of alcohol precipitated, dried and ground diphtheria bacilli.

It therefore seems improbable that it will be possible either for practical purpose or experimentally to concentrate diphtheria antitoxins by producing precipitins to the proteins of the bacteria concerned in the antitoxin producing animals.

LYSINS

Sensitizers or amboceptors

Under this heading we propose to discuss those substances in immune serum which produce visible solution of a cellular antigen. We shall discuss the substances in serum that protect from injection with the antigenic micro-organism separately without prejudice as to their relation to these lysins, sensitizers or amboceptors. It is known from the work of Camus and Gley (12) and of Bordet (13) that when the normal or artificial hemolysin in a serum of species A is mixed with the serum of an animal immunized against serum A, the hemolytic power is neutralized. Owing to the conditions of the experiment in the light of subsequent information the question might well be raised as to whether this inhibition might not be due to the formation of a precipitate in the mixture rather than to the presence of real antihemolysins (antisensitizers, antiamboceptors). The subsequent work of Pfeiffer and Friedberger (14) and of Bordet (15) showed, however, that an antihemolysin or an antibacteriolysin will not only neutralize the effect of a hemolysin or bacteriolysin, but will actually "cure" blood cells or vibrios that have already been treated with heated immune serum (sensitized) and washed. These facts would seem to demonstrate the existence of actual antisensitizers rather than inhibition of sensitizer action by a specific precipitate.

We find, however, no actual test as to the fate of the lysin when in the presence of precipitate formation apart from the work of Zebrowski (16), who found that the precipitinogen does not carry down the hemolysin. It is important not only to confirm this statement, which was based on somewhat indirect evidence, but also to test the reverse of the reaction, namely the fate of the lysin that accompanies a precipitin. In the case of reactions

with pneumococcus serum, at least, the fate of the protective bodies differs diametrically in accordance with whether it is associated with the precipitin or with the precipitinogen and for this reason we have endeavored to test both types of precipitate formation so far as possible.

Bacteriolysins

In order to test the effect of precipitate formation on bacteriolysins we immunized rabbits against *Sp. cholerae asiaticae* the classical micro-organism for demonstration of Pfeiffer's phenomenon. Three rabbits were given three or four injections of killed, and then four or five of living twenty-four-hour cultures of *Sp. cholerae asiaticae* in doses beginning in both instances with one-fifth and increasing to three-fifths of an agar slant. They suffered no appreciable loss of weight and were bled from seven to ten days after the last injection.

The serum of rabbit 3, heated to 56°, was found to give a clear cut, granular transformation (Pfeiffer's phenomenon) of fresh cholera vibrios in a dilution of 0.01 cc. with guinea pig alexin. It also agglutinated the micro-organism in considerably higher dilution, a matter to which reference will be made in another place.

This rabbit anticholera serum also contains precipitins for extracts of the cholera spirillum. Although it is relatively an easy matter to obtain some grade of specific reaction of this type, securing the maximal reaction may be more complicated and dependent on more factors than the simple question of dosage. Pick (17) has shown that the precipitin reactions between antityphoid and anticholera sera and their respective antigens, demonstrate that the micro-organisms contain two definite precipitinogenic substances, one contained in the filtrate from old bouillon cultures and the other obtainable from fresh cultures of the bacteria autolysed in sodium chlorid solution. These antigens designated as A and K, differ chemically and in particular call forth the action of corresponding precipitins in the antiserum so that a serum which has produced the maximum precipitate with antigen A would still react as well as whole serum with K, al-

though it no longer reacts with A. Our work with pneumococcus indicates that the concentration of protective bodies in their entirety is much more likely when the maximum precipitate has been obtained. For this reason we prepared cholera extracts in two different ways, corresponding, although not identical with the method prescribed by Pick.

1. Cholera extract no. 1 was prepared by growing the organism in bouillon for four weeks in the incubator and passing through a Berkefeld filter.

2. Cholera extract no. 2 was made by suspending forty-eight-hour cultures of *Sp. Cholerae* in saline, precipitating with 3 volumes of acetone, centrifugalizing, and drying the bacterial sediment. This sediment was ground in an agate mortar and suspended in saline in the proportion of 1 mgr. to 1 cc. of carbolated saline (0.5 per cent). This mixture was boiled for three minutes and re-centrifugalized and the clear supernatant fluid is used for precipitin reaction.

The serum of rabbit 1 gives a good precipitin reaction with both of these extracts of cholera vibrios and with equal intensity. 0.05 cc. of either filtrate produced a marked flocculation with 0.5 cc. of serum. Heating the serum to 56° apparently does not reduce the precipitin potency.

Mixtures were then made with filtrates in the following proportions:

1. Rabbit anticholera serum no. 3, 56° 5 cc. + broth filtrate cholera, 2 cc.
2. Rabbit anticholera serum no. 3, 56° 5 cc. + boiled extract cholera, 2 cc.
3. Rabbit and cholera serum no. 3, 56° 5 cc. + bouillon, 2 cc.

These mixtures after shaking were left over night at room temperature. Marked precipitates settled out in tubes 1 and 2. After centrifugalizing these two tubes the same materials were added to the supernatants as before, i.e., broth filtrate to no. 1, boiled antigen to no. 2, and bouillon to no. 3. Slight further precipitation occurred in 1 and 2.

The supernatants 1 and 2 were then tested with the crossed bacterial extracts as follows:

A. Supernatant no. 1, 0.5 cc. + boiled extract 0.5 cc.

B. Supernatant no. 2, 0.5 cc. cc + broth filtrate 0.5 cc.

No precipitate was evident on standing indicating that with our sera and antigens no separate precipitins and precipitinogens can be found as did Pick.

The supernatant fluids in which the maximum precipitates had been found, were then tested for their ability to produce Pfeiffer's phenomenon as compared with the original diluted serum (tube 3).

TABLE 1

*Pfeiffer's phenomenon with rabbit anticholera serum before and after precipitation.
Mixtures as follows:*

1. Supernatant 1, 0.1*	+ guinea pig alexin 5% 1 cc.**	+ cholera vibrios 0.5 cc.†
2. Supernatant 1, 0.02	+ guinea pig alexin 5% 1 cc.	+ cholera vibrios 0.5 cc.
3. Supernatant 1, 0.01	+ guinea pig alexin 5% 1 cc.	+ cholera vibrios 0.5 cc.
4. Supernatant 1, 0.002	+ guinea pig alexin 5% 1 cc.	+ cholera vibrios 0.5 cc.
5. Supernatant 1, 0.001	+ guinea pig alexin 5% 1 cc.	+ cholera vibrios 0.5 cc.
6. Supernatant 2, 0.1*	+ guinea pig alexin 5% 1 cc.**	+ cholera vibrios 0.5 cc.†
7. Supernatant 2, 0.02	+ guinea pig alexin 5% 1 cc.	+ cholera vibrios 0.5 cc.
8. Supernatant 2, 0.01	+ guinea pig alexin 5% 1 cc.	+ cholera vibrios 0.5 cc.
9. Supernatant 2, 0.002	+ guinea pig alexin 5% 1 cc.	+ cholera vibrios 0.5 cc.
10. Supernatant 2, 0.001	+ guinea pig alexin 5% 1 cc.	+ cholera vibrios 0.5 cc.
11. Fluid 3.....0.1*	+ guinea pig alexin 5% 1 cc.**	+ cholera vibrios 0.5 cc.†
12. Fluid 3.....0.02	+ guinea pig alexin 5% 1 cc.	+ cholera vibrios 0.5 cc.
13. Fluid 3.....0.01	+ guinea pig alexin 5% 1 cc.	+ cholera vibrios 0.5 cc.
14. Fluid 3.....0.002	+ guinea pig alexin 5% 1 cc.	+ cholera vibrios 0.5 cc.
15. Fluid 3.....0.001	+ guinea pig alexin 5% 1 cc.	+ cholera vibrios 0.5 cc.
16. NaCl.	+ guinea pig alexin 5% 1 cc.	+ cholera vibrios 0.5 cc.

* To volume of 1 cc. in 0.85 per cent saline.

** Fresh mixed twenty-four hour serum from two guinea pigs.

† From a twenty-four hour agar slant of sp. cholerae suspended in 5 cc. saline.

These two mixtures were incubated at 37.5°C. for two hours and smears made from each tube were stained with dilute carbol fuchsin or toluidin blue. A complete granular degeneration of practically all the vibrios was the criterion of a positive Pfeiffer's phenomenon. The reaction was complete in the following tubes; 1, 2, 3, 6, 7, 8, 11, 12, 13 and absent in the remaining tubes. This experiment shows clearly that the maximum precipitate in an

anticholera serum from the rabbit produced by adding extracts of cholera spirilla has no effect on the content of the serum in its specific lysins. The experiment was repeated with identical results with the serum of rabbit 3. The serum of rabbit 2, although containing agglutinins and lysins, had no precipitins for the extract.

Bacteriolysins in combination with precipitinogen

For the purpose of this and other experimental combinations a goat was immunized by repeated intravenous injections of normal rabbit serum. This goat serum gave precipitin reactions readily with normal or immune serum from the rabbit. Rabbit anticholera serum was precipitated by this goat antirabbit serum as shown in the following protocol:

1. Rabbit anticholera serum 56° 0.5 cc. + goat antirabbit serum, 56°, 4 cc.
2. Rabbit anticholera serum 56° 0.5 cc. + NaCl, 4 cc.

On standing for four hours at room temperature a marked precipitate was present in 1. To the supernatant of tube 1 and to tube 2 was then added an additional 0.5 cc. of the heated anticholera serum. A trace more precipitate had formed in no. 1 on standing over night. After recentrifugalizing a third addition of 0.5 cc. of the anticholera serum was made and four hours later, when no precipitate was found, the two fluids were tested in descending doses for their ability to produce Pfeiffer's phenomenon in the manner just described.

The precipitated anticholera serum produced a complete Pfeiffer's in a dilution of 0.025¹ cc. and the serum simply diluted went to the same dilution but no further.

No bacteriolysin then is brought down through association with a precipitinogen.

Hemolysins

We have already mentioned that Zebrowski (16) found the hemolytic sensitizer unaffected by precipitate formation. His

¹ Reckoned from original serum volume.

experiment consisted in adding the serum of a rabbit immunized against dog serum to the serum of a dog that had been immunized against bovine red blood cells. A precipitate resulted and the supernatant fluid contained a hemolysin for bovine red blood cells as active as before. In this combination the lysin is present in the precipitinogenic serum. It seemed wise to test the reverse phenomenon and also to add other examples to these observations of Zebrowski.

A. Hemolysins in combination with precipitin

One series of rabbits was immunized against *unwashed* guinea pig blood by repeated intravenous injections and another series against *unwashed* sheep blood, the object in using unwashed corpuscles being to produce a precipitin as well as a hemolysin. After immunization it was found that the sera of the rabbits immunized against guinea pig blood contained (a) lysins for washed guinea pig corpuscles; in which case rabbit alexin (complement) was employed; (b) agglutinins for guinea pig corpuscles; (c) precipitins for guinea pig serum. The sera of rabbits immunized against unwashed sheep blood showed the presence of lysins and precipitins but no agglutinins.

On producing various degrees of precipitation by employing various amounts of the antigenic serum it was found invariably that the subsequent supernatant fluids contain as much hemolysin as before the treatment in both combinations. This may be illustrated by the following experiment:

The following mixtures were made:

A. Serum rabbit antish sheep blood no. 7, 56° 2 cc. + sheep serum 56°, 0.03 in 3 cc.

B. Serum rabbit antish sheep blood no. 7, 56° + NaCl, 3 cc.

A voluminous precipitate was formed in A which was allowed to stand at 37° for one hour. No precipitate in B. Fluid A was centrifugalized and the supernatant titrated for its content in hemolytic sensitizer in comparison with B employing guinea pig alexin in a dose of 0.05 cc. and 1 cc. of washed 5 per cent

sheep blood, with the resultant hemolysis shown in table 2. In another experiment three successive doses of the sheep serum as above (total 0.09 in 9 cc.) was added to the hemolytic serum with the faintest possible precipitate on the second addition and none on the third. There was no difference from the result with the hemolytic titer over that to be described.

TABLE 2

	HEMOLYSIS TWO HOURS
1. Supernatant A..0.002* in 1 cc. + washed sheep blood 5% 1 cc. + alexin 5% 1 cc.	Complete
2. Supernatant A..0.0002 in 1 cc. + washed sheep blood 5% 1 cc. + alexin 5% 1 cc.	Complete
3. Supernatant A..0.0001 in 1 cc. + washed sheep blood 5% 1 cc. + alexin 5% 1 cc.	Partial
4. Supernatant A..0.00005 in 1 cc. + washed sheep blood 5% 1 cc. + alexin 5% 1 cc.	None
5. Fluid B.....0.002 in 1 cc. + washed sheep blood 5% 1 cc. + alexin 5% 1 cc.	Complete
6. Fluid B.....0.0002 in 1 cc. + washed sheep blood 5% 1 cc. + alexin 5% 1 cc.	Complete
7. Fluid B.....0.0001 in 1 cc. + washed sheep blood 5% 1 cc. + alexin 5% 1 cc.	Partial
8. Fluid B.....0.00005 in 1 cc. + washed sheep blood 5% 1 cc. + alexin 5% 1 cc.	None
9 NaCl..... 1 cc. + washed sheep blood 5% 1 cc. + alexin 5% 1 cc.	None

* Reckoned from original volume of serum.

Other experiments show that the washed precipitate from the original combination of sera after solution in normal rabbit serum has no hemolytic activity for sheep corpuscles.

B. Hemolysins in combination with precipitinogen

Combinations of goat antirabbit serum 4 cc. and either rabbit antiginea pig serum or rabbit antisheep serum were made, the precipitate allowed to form at 37° and the supernatant fluid tested for hemolysins as compared with a similar dilution of the hemolytic rabbit serum in saline solution. The results of such experiments may be summarized as follows:

- A. Rabbit antishoop serum 56° 1 cc. + goat antirabbit 56°, 4 cc.
- B. Rabbit antishoop serum 56° 1 cc. + NaCl, 4 cc.

Hemolytic titer (complete hemolysis) of supernatant of A after precipitation with 1 cc. of 5 per cent pig guinea alexin and 1 cc. of a 5 per cent suspension of washed sheep blood was 0.0000625 and *precisely the same with fluid B*.

In a similar experiment with rabbit antiginea pig serum, 56°, the complete hemolytic dose for 1 cc. 5 per cent washed guinea pig blood, employing 1 cc. of a 10 per cent solution of fresh rabbit serum as alexin, was found for both precipitated and simply diluted serum to be 0.005 cc. In both these experiments a control supernatant fluid from a mixture of normal rabbit serum and goat antirabbit serum was found to have no hemolytic effect on the corpuscles employed.

It would seem evident from these experiments that hemolysins and bacteriolysins from the rabbit, at least in the combinations employed, are not affected by natural association with a precipitinogen or precipitin that may enter into a precipitin reaction.

AGGLUTININS

Some data are already at hand concerning the fate of agglutinins in the precipitin reaction. We have already described the observations of the pneumococcus agglutinins in the precipitate that collects the protective bodies (1). We found it relatively difficult to deprive the serum entirely of its agglutinins, which bodies moreover are present in relatively weak concentration. Pick (17) found that the agglutinins in antityphoid and anti-cholera serum from the horse were not brought down in the precipitin reactions he described, *i.e.*, when the agglutinins are associated with the serum containing the precipitin. Similar results were found by Kraus and Eisenberg and by Radziewsky (18). Kraus and Pribram (7) found that agglutinins were sometimes brought down and sometimes not. V. Eisler and Tsuru that normal hemagglutinins in bovine serum were usually removed by precipitation whereas immune bacterial agglutinins sometimes were and sometimes were not. Landsteiner and Prasez (19)

found normal hemagglutinins and bacterial agglutinins brought down in precipitate formation.

The question then seems an open one and it seemed important to investigate in particular the behavior of agglutins in accordance with their association with both precipitin and precipitinogen.

Bacterial agglutinins

The rabbit anticholera serum that we have already described agglutinates as well as dissolves cholera spirilla and the former of course without the adjuvant alexin. In the experiment described under bacteriolysins (table 1) it was found that the limits of agglutinin titer with the precipitated anticholera sera (supernatants 1 and 2) and the simply diluted anticholera serum (fluid 3) was exactly the same, namely 0.002 cc. This result was uniformly obtained in other experiments.

In the next experiment (p. 91) in which the agglutinating serum accompanied the precipitinogen, the precipitated serum gave an agglutination titer of 0.001 cc. and the diluted serum of 0.0005 cc., a slight difference and within the limits of experimental error.

Rabbits and goats were immunized against the typhoid bacillus by repeated intravenous injections of a mixed vaccine prepared from six recently isolated strains of *B. typhosus*. A killed (carbolic acid 0.5 per cent) polyvalent vaccine was first used, followed by a living vaccine.

An experiment may be given showing the fate of the agglutinin when the rabbit antityphoid serum employed was used either as a precipitinogen or as a precipitin. Preliminary experiments with this serum showed that precipitates could be readily produced with unheated rabbit antityphoid serum, whereas when the serum was heated, little or no result was produced on adding extracts of the typhoid bacillus. Two solutions of precipitinogen were tested:

A. A solution of dried typhoidin (20). This is prepared from a ten-day culture of *B. typhosus* in 5 per cent glycerine bouillon evaporated to one-tenth volume at 56° and precipitated with

twenty volumes absolute alcohol; washed with absolute alcohol and ether and dried. The resulting powder is dissolved in an amount of carbolated saline solution equivalent to the original bouillon before evaporation, *i.e.*, about 6 grams to the liter.

B. An extract of typhoid bacilli prepared by precipitating the organisms from saline by equal parts of absolute alcohol. These killed bacteria are dried in partial vacuum over H_2SO_4 and ground. The dried ground bacteria are then extracted in carbolated saline and the clear supernatant fluid used as a precipitinogen after removing the sediment. A better precipitate is produced when this supernatant extract is boiled.

Experiments show that the precipitation of the fresh antityphoid serum with either of these extracts removes the precipitin for the other extract and in the doses used in the following experiment the totality of precipitin is formed by a single addition to the antigen. In the final experiment the following mixtures were prepared:

1. Unheated rabbit antityphoid serum 1 cc. + NaCl, 4 cc.
2. Unheated rabbit antityphoid serum 4 cc. + boiled typhoid extract b., 1 cc.
3. Unheated rabbit antityphoid serum 1 cc. + goat antirabbit serum 56°, 4 cc.

These mixtures stood at 37°C. for two hours and then over night at ice box temperature. Precipitates were formed in 2 and 3. The agglutinin titer of the supernatant fluids of 2 and 3 and of fluid 1 was then determined by adding to decreasing doses of the serum dilutions in 1 cc. three drops of a stock formalinized suspension of *B. typhosus* (macroscopic method) and reading on the following day after allowing them to stand at room temperature. The titer of each dilution reckoned from the original agglutinating serum employed was the same, namely 1 to 10,000.

In a similar experiment antityphoid serum from goats was precipitated by adding four successive doses of the boiled typhoid extract "B" to the sera with appropriate controls. The maximum precipitate was produced in the first addition but subsequent additions produced traces of precipitate. Twenty-four

hours were allowed to elapse between each addition and the titer of each serum dilution determined with the following result:

TABLE 3

Agglutinin titer of antityphoid sera from goats with and without previous precipitate formation

1. Serum of Goat C diluted in saline titer.....	1-800
2. Serum of Goat C after precipitation titer.....	1-400
3. Serum of Goat D diluted in saline titer.....	1-6400
4. Serum of Goat D after precipitation titer.....	1-3200

This last experiment does indicate a slight reduction of agglutinin titer after precipitation which, however, is within the limit of experimental error when one considers that successive dilutions were made by multiplying by two so that the results express the difference of a single tube only in the series. The general conclusion is that the immune bacterial agglutinins tested were not affected by the formation of a precipitin reaction whether the immune serum was associated with precipitin or precipitinogen.

Hemagglutinins

The properties of the sera of rabbits immunized against guinea pig blood have already been discussed under the heading of hemolysins. Such sera agglutinate guinea pig red blood cells as well as hemolyse them. In precipitated serum prepared as described either by adding heated guinea pig serum 56°, to rabbit antiguinea pig serum 56°, or by adding rabbit antiguinea pig serum 56° 1 part to Goat-anti-rabbit serum 56°, 4 parts, it is found that the hemagglutinin is present in as high dilutions as in the control with saline. We conclude therefore that immune hemagglutinins as well as bacterial agglutinins remain unaffected by precipitation.

FIXATION ANTIBODIES

Gengou (21) showed some years ago that soluble antigens, serum, fibrinogen, milk and the like, when mixed with their antisera, give rise to alexin fixation, the phenomenon previously described by Bordet and Gengou (22) with cellular antigens. As

one of the writers was first to show this phenomenon when it takes place in a mixture of serum and its antiserum is apparently caused by the specific precipitate which usually occurs in such a mixture. It has since been shown that this fixation reaction may occur in absence of visible precipitate, a fact which does not of necessity rule out the possibility of an invisible precipitate or change in colloidal condition essentially similar to the precipitin reaction. Further study (4) of combinations in which precipitate formation was coincident with fixation led us to the conclusion that the precipitate does not fix *per se*, but owing to the presence of a combination of soluble antigen and antialbuminous sensitizer adherent to it. This conception which we still hold represents simply further experimental evidence of Gengou's original proof of the existence of antialbuminous sensitizers.

Extracts of bacteria plus corresponding antibacterial sera fix alexin as rapidly as the whole bacteria plus antiserum. In combinations such as these Haendel (23) noted the parallelism between fixation and precipitation. Toyosumi (24) decided that fixation by vibrios and specific antiserum is produced by the precipitate formed and not by a union of the cell with its sensitizer. Crendiropoulo (25) in a similar set of experiments found that fixation is due to an extra cellular mixture of an extractive from the vibrios with the serum and not to the lytic sensitizers or amboceptors fixed on the cell. The results agree with those of Neufeld and Hüne (26) and Neufeld and Haendel (27) who conclude that special antibodies, "Bordet's antibodies," and not the bacteriolytic amboceptors are responsible for fixation in these combinations. The further confirmation of these latter experiments becomes evident when we describe the fixing properties of certain of the precipitates we have already described.

The technic we employ in alexin fixation experiments has been described in previous publications (4) and need not be repeated here. It may suffice to add that in the summary following the results given as indicating the locus of the fixing complex in supernatant fluid or precipitate do not in all instances indicate that complete fixation, that is complete absence of hemolysis, was obtained. The differences are relative, not absolute, as

the interest has been to determine whether the fixation complex lay in one part of the precipitated mixture rather than the other. In most of the instances given the experimental results were repeated at least once. In the combinations to be given precipitates were produced in one of two ways; either by adding a serum to its antiserum, or by adding a bacterial extract to its antiserum. In all instances the maximum precipitate was produced by successive doses of the precipitinogen. The results of many experiments and several combinations are summarized in the following table.

TABLE 4
Residence of fixation complex in a specific precipitin combination

	COMPLEX
1. Rabbit antishoop serum + sheep serum...	Fixation complex in precipitate.
2. Rabbit antiguinea pig serum + guinea pig serum.....	Fixation complex in precipitate.
3. Rabbit antiegg serum + egg.....	Fixation complex in precipitate.
4. Rabbit antihorse serum + horse antipneumococcus serum.....	Fixation complex in precipitate.
5. Rabbit antihorse serum + horse serum...	Fixation complex in precipitate.
6. Goat anti-rabbit serum + normal rabbit serum.....	Fixation complex, supernatant.
7. Goat anti-rabbit serum + rabbit anticholera.....	Fixation complex, supernatant.
8. Goat anti-rabbit serum + rabbit antishoop.....	No fixation in spite of voluminous precipitate.
9. Horse antipneumococcus serum + extract of Pneumococcus.....	Fixation complex in supernatant.
10. Goat antityphoid + extract of B. typhosus	Fixation complex in supernatant.
11. Rabbit anticholera + extract sp. cholerae.	Fixation equal in precipitate and supernatant.

It will be seen from this table that the majority of combinations of serum and antiserum fix alexin in the precipitate fraction as was expected from previous work. The exceptions are with antisera from the goat where fixation was produced by the supernatant part or in one instance where there was no fixation at all. It should be noted that in these combinations the precipitate, although eventually considerable, was very slowly formed,

an observation which would agree with certain results of Dean (28). In the mixtures of bacterial antiserum and extracts of bacteria, the fixing property seem to lie largely in the supernatant fluids.

We regard these observations again as indicating that the fixation complex is separable from the precipitate although frequently adherent to it.

PROTECTIVE ANTIBODIES

The last observations on fixation antibodies are of interest in their relation to the behavior of the actual curative or protective bodies in serum. As we have shown before and repeated in the above connection, the entirety of the protective power of antipneumococcus serum from the horse is brought down with the precipitate whereas the fixation antibodies are present in the supernatant. Conversely the precipitate produced by the combination rabbit antihorse serum and horse antipneumococcus serum fixes alexin but the protective bodies against pneumococcus infection are in the supernatant. There seems then direct proof that the protective bodies (tropins or lysins) are separate from the fixation antibodies in antipneumococcus serum. Similar results are indicated by some experiments on the protective value of rabbit anticholera serum in mice. The supernatant fluid contains all the protecting bodies although the fixation is produced by both the precipitate and supernatant fluids. Other combinations of this sort may be worthy of further consideration.

In an attempt to parallel the pneumococcus work more closely with similar antisera, we have met with entire failure. Two specimens of polyvalent antimeningococcus serum from the horse, one given us by Dr. Flexner and one from the Cutter Analytic Laboratory in Berkeley, both failed to give precipitates with extracts of the meningococcus prepared as for the pneumococcus work. Antistreptococcus serum from the Cutter Laboratory failed to yield precipitates with extracts of some of the strains of streptococcus used for immunization.

SUMMARY

Previous work has shown that at least certain antibodies may be brought down in the course of a specific precipitin reaction. Antitoxins (Dehne & Hamburger, Weill-Hallé and Lemaire); fixation antibodies (Gay); and the protective bodies of anti-pneumococcus serum (Gay and Chickering) are instances of this phenomenon. It seemed advisable to undertake a more systematic study of the several types of antibodies to learn how frequent this occurrence may be. It would be of great service to separate out antibodies in this manner as they could then be studied in a condition relatively free from other proteins and certain very practical results might be obtained with curative sera.

Attention is drawn to the fact that in the combinations noted above in which successful results have been obtained, the antibody adherent to the precipitate may have been originally associated with either the precipitinogen or with the precipitin. This is a matter not only of theoretic interest, but of practical importance in concentrating antisera. For this reason we have, so far as possible, endeavored to trace the antibody when it is associated with both factors in the precipitin reaction.

Our results have, for various reasons, been entirely negative, at least in so far as separating out antibodies is concerned. They seem, however, of value as being the first attempt at a systematic study of this kind and as saving others from fruitless endeavor.

The previous method of bringing down antitoxins with the precipitin has been shown in those instances in which the antitoxic serum served as the precipitinogen in the reaction. We have attempted to reverse the phenomenon by producing both antitoxins and precipitins to the diphtheria bacillus in rabbits and horses. Our results hitherto have been without result, owing to the fact that we have not succeeded in producing precipitins in these animals.

Experiments with various combinations have shown that both bacteriolysins and hemolysins when associated either with the precipitinogen serum or with the precipitin serum are not

brought down in the formation of the precipitate. Similar negative results were obtained with artificial bacterial agglutinins and hemagglutinins.

Experiments on the fate of the fixation antibodies in the precipitin reaction shown, in general, that when the precipitate is produced by adding serum to its antiserum the fixation complex is present in the precipitate. This is by no means universal, as others have pointed out, and the fixation complex may lie in the supernatant fluid, or in both the supernatant fluid and the precipitate. The difference in results would seem to depend somewhat upon the rapidity of the precipitate formation without relation to its actual intensity. When bacterial extract is added to an immune serum the fixation complex seems usually present in the supernatant fluid.

In certain combinations it seems definitely shown that the fixation complex is present in that fraction (supernatant or precipitate) in which the protective bodies are absent. Thus in the case of pneumococcus precipitate produced by adding the extract of pneumococcus to antiserum from the horse, the protective bodies are present in the precipitate and the fixation complex is present in the supernatant fluid. The exact reverse is true in a combination of rabbit antihorse serum and horse antipneumococcus serum.

We have not been able to find precipitins in the antisera to meningococcus and streptococcus from the horse which are used for curative purposes, and which were produced in a manner similar though perhaps not identical to the pneumococcus serum which we previously investigated.

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KIDNEY LESIONS IN CHRONIC ANAPHYLAXIS

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The subject of the tissue lesions in anaphylaxis, and especially in chronic anaphylaxis, has attracted the attention of but few investigators. The phenomenon of Arthus (1) is well known. He found that the repeated subcutaneous injection of small amounts of horse serum into rabbits at intervals of five to seven days eventually produced infiltration, edema, sterile abscesses, and gangrene of the part, even though the injections were given in different locations. This effect was noted without the definite production of anaphylactic shock. Gay and Southard (2) studied the lesions of acute anaphylaxis. They reported finding three types of lesions. The first was hemorrhage, and in many cases this was plainly due to lesions of capillary endothelium. The second was fatty changes, principally in capillary endothelium, but also noted in voluntary muscle, heart muscle, nerve fibres, and in some epithelial structures. The third was a "species of alteration" in the large nerve cells of the spinal cord and bulb, but this was stated to be within the limits of error of staining, and probably secondary to nerve fiber changes. Longcope (3) states that he has frequently observed similar changes in his own animals, but Rosenau and Anderson (4) repeating the work of Gay and Southard, considered the hemorrhages of but little importance, and were unable to find the other lesions mentioned. Later Gay and Southard (5) studied the effect of repeated anaphylactic shock, but were unable to produce chronic lesions. Schlecht and Schwenker (6) repeating and extending the work of Friedberger (7), who produced a "sterile pneumonia" by intratracheal sprays of horse serum in sensitized animals,

found proliferation of alveolar epithelium and a fibrinous exudate containing many eosinophiles within the alveoli. Dale (8) showed that the reaction of anaphylaxis is essentially cellular by perfusing the uterus of a sensitized guinea pig for some time, and showing that it still reacted typically to antigen. It might be inferred from this that cellular lesions might be expected in anaphylaxis. Worzekowsky and Kundratitz (9) and Beneke and Steinschneider (10) report granular and waxy changes and fragmentation of skeletal and heart muscle in acute anaphylaxis. Longcope (11) reports the results of repeated anaphylactic shock induced in dogs, cats, rabbits, and guinea pigs by the injection of horse serum and egg-white, separately and in combination. He reports finding chronic changes in peritoneum, lungs, liver, heart, and kidney. The most frequent and striking changes were in the kidney. These changes consisted primarily in a localized necrosis of tubular epithelium, accompanied by round celled infiltration, which, in some cases, became so marked as to obscure the kidney structure. In the most marked cases the glomeruli showed a fibrous thickening of the capsule, proliferation of capillary endothelium, with occasionally complete hyalin degeneration of the tuft, and sometimes proliferation of capsular epithelium. Those cases dying from acute anaphylaxis showed cloudy swelling of the cells of the convoluted tubules, and extreme congestion of the glomeruli. In a later paper Longcope (12) describes heart lesions obtained in a similar manner in rabbits and other animals. These lesions consist of focal necroses, round celled infiltration, and later deposition of scar tissue, so as to produce the appearance of interstitial myocarditis.

The present paper deals with the results obtained by attempting to produce kidney lesions by inducing repeated anaphylactic shock in animals. Rabbits and guinea pigs were used at first, but for various reasons the rabbits were unsatisfactory, therefore the results reported here are based upon the findings in guinea pigs alone. Beef serum and egg-white were used as antigens. All of the injections were given intraperitoneally. Sensitizing doses ranged from 0.001 to 1 cc. The interval be-

tween the sensitizing dose and the first toxic dose varied from ten to twenty-five days but it was usually not less than twenty days. The toxic doses ranged as high as 10 cc., the serum being used undiluted, and the egg-white in a 50 per cent solution, but the attempt was made to give in any given case the smallest amount that would produce a marked anaphylactic shock. Some of the animals received as many as 19 toxic doses, but the average was 9. Some of the animals were under observation for longer than eight months, but the average was about four months. Those animals that died with acute anaphylaxis were posted at once. Others were killed at varying intervals up to eight weeks after the last injection. Organs were fixed in formalin and Zenker's fluid and stained with hematoxylin and eosin, and some were run through by the Marchi method as well.

It is, of course, difficult to so regulate the dose as to give an animal repeated anaphylactic shocks and still keep it alive. If the dose is too large the animal dies; if the dose is too small, no shock is produced, and the animal becomes refractory to anything but very large doses, and it is not practicable to give guinea pigs intraperitoneal injections much larger than 10 cc. After a single large dose had been given the animal was usually completely refractory for several weeks. For convenience the shocks were classified as mild, moderate, and severe. In a mild shock the animal would at first be very quiet, then become uneasy and scratch itself, especially about the head. There was frequently twitching of the ears. The hair about the head and neck, and sometimes over the whole body, became ruffled, respirations were deeper than normal and urination and defecation were noted. In a moderate shock, in addition to the above symptoms, the animal would lie on its side and appear prostrated. It would not resist handling, and would frequently show muscular twitching. Respirations were labored. In a severe shock these symptoms would be exaggerated, and last for a longer time. The animal would appear drowsy and almost comatose, and would frequently show convulsive movements. Respirations were often rapid and shallow.

The urine was not examined systematically, but a few speci-

mens were examined, and no albumin was found. None of the animals lost weight or appeared to suffer in general health during the experiment. Indeed, all of the animals gained rather rapidly in weight, but this was probably due to the fact that young animals were used for the experiments in nearly all cases.

Three types of controls were used: normal animals; animals dying from a single shock (to show that the lesions were not the result of acute anaphylaxis) and animals receiving many (12 to 15) doses of protein so spaced as to avoid producing anaphylaxis (to show that the lesions were not the result of the protein itself, but of the shock produced.)

Some typical protocols are given:

Guinea pig 5. Male, 300 grams

November 20, 1914. Injected 1 cc. albumin solution intraperitoneally.
December 1. Injected 0.7 cc. albumin solution. Moderate reaction.
December 4. Injected 0.7 cc. albumin solution. No reaction.
December 8. Injected 1 cc. albumin solution. No reaction.
December 15. Injected 1 cc. albumin solution. Slight reaction.
December 18. Injected 1 cc. albumin solution. Slight reaction.
December 21. Injected 1 cc. albumin solution. Slight reaction.
December 24. Injected 1 cc. albumin solution. Slight reaction.
December 28. Urine examined: no albumin.
December 29. Found dead. Urine removed from bladder showed no albumin or casts. Spleen is large, other organs appear normal grossly.

Microscopic examination: Kidney shows slight hyperemia. A few small areas of round celled infiltration are scattered diffusely over the cortex. The convoluted tubules show well marked vacuolation, necrosis, and desquamation of epithelial cells. The tubules of Henle's loop and the glomeruli show no lesions.

Guinea pig 11. Female, 415 grams

December 15, 1914. Injected 1 cc. albumin solution intraperitoneally.
December 28. Injected 0.5 cc. albumin solution. Reaction slight.
December 31. Injected 0.75 cc. albumin solution. Reaction slight.
January 5. Injected 1 cc. albumin solution. Reaction slight.
January 8. Injected 1 cc. albumin solution. No reaction.
January 12. Injected 1.5 cc. albumin solution. Slight reaction.

- January 15. Injected 2 cc. albumin solution. Very slight reaction.
January 19. Injected 3 cc. albumin solution. No reaction.
January 22. Injected 4 cc. albumin solution. No reaction.
January 26. Injected 6 cc. albumin solution. No reaction.
January 29. Injected 10 cc. albumin solution. No reaction.
February 5. Injected 2 cc. albumin solution. Moderate reaction.
February 13. Injected 2 cc. albumin solution. Slight reaction.
February 16. Injected 0.5 cc. beef serum.
February 19. Injected 4 cc. albumin solution. No reaction.
March 2. Injected 3 cc. albumin solution. No reaction.
March 17. Injected 4 cc. albumin solution. Severe reaction.
March 23. Injected 6 cc. albumin solution. Slight reaction.
June 17. Injected 1 cc. albumin solution. No reaction.
June 18. Injected 3 cc. albumin solution. No reaction.
June 21. Injected 8 cc. albumin solution. No reaction.
August 14, 1915. Killed.

Microscopic examination: A slight amount of hemorrhage and hyperemia noted. A few small areas of round celled infiltration noted in the cortex. Necrosis is fairly well marked in the epithelial cells of the proximal convoluted tubules, but vacuolation is not present. Most of the glomeruli show a very marked proliferation of the capillary endothelium of the tuft, and in a few glomeruli the capsular epithelium has also undergone proliferation.

Guinea pig 31. Female, 375 grams

- April 15. Injected 0.5 cc. beef serum intraperitoneally.
May 26. Injected 0.001 cc. albumin solution.
June 15. Injected 0.001 cc. albumin solution. No reaction.
June 17. Injected 0.03 cc. albumin solution. Slight reaction.
June 18. Injected 0.1 cc. albumin solution. Slight reaction.
June 21. Injected 0.3 cc. albumin solution. Slight reaction.
June 22. Injected 1.5 cc. albumin solution. Moderate reaction.
June 25. Injected 5 cc. albumin solution. Severe reaction; died in 1 hour. Posted at once.

Post mortem findings: Marked injection of stomach, intestines, and adrenals. Left adrenal and left kidney larger than normal. Spleen large and nodular, lungs puffy, heart very red.

Microscopic examination: Hyperemia very marked; slight amount of hemorrhage; many areas of round celled infiltration scattered diffusely

throughout the cortex. There is marked vacuolation and a moderate amount of necrosis in the epithelial cells of the convoluted tubules. The cells of the ascending limb of Henle's loop show some vacuolation and apparent desquamation. Most of the glomeruli show swelling and proliferation of the endothelial cells of the capillaries of the tuft.

Guinea pig 19. Male, 240 grams

February 5. Injected 1 cc. egg albumin solution intraperitoneally.
February 13. Injected 0.5 cc. beef serum.
February 26. Injected 0.5 cc. albumin solution. Slight reaction.
March 2. Injected 0.5 cc. albumin solution. No reaction.
March 5. Injected 2 cc. beef serum. Slight reaction.
March 9. Injected 1 cc. albumin solution. Slight reaction.
April 13. Injected 1 cc. albumin solution. Moderate reaction.
April 15. Injected 2 cc. albumin solution. Slight reaction.
May 7. Injected 5 cc. serum. Slight reaction.
May 19. Injected 4 cc. albumin solution. Slight reaction.
May 23. Injected 7 cc. serum. Slight reaction.
May 25. Injected 7 cc. albumin solution. No reaction.
June 28. Bled to death.

Microscopic examination: No hyperemia; a few small areas of hemorrhage. Many small areas of round celled infiltration especially about glomeruli. Most glomeruli show marked proliferation of capillary endothelium. Some show proliferation of the capsular epithelium. In some glomeruli the tuft has been entirely replaced by connective tissue. Most of the convoluted tubules are normal, but some show necrosis and desquamation, and a few show vacuolation of the epithelium. The epithelial cells of the tubules of Henle's loop show no lesions. Edema was noted in the interstitial tissue of the papilla.

Friedberger and Mita (13), Biedl and Kraus (14), Pearce and Eisenbray (15), Pepper and Krumbhaar (16) and others have shown that in anaphylaxis there is a marked lengthening of the coagulation time of the blood—in dogs amounting practically to prevention of coagulation. Jobling and Petersen (17) in injecting guinea pigs with "serotoxins" (normal guinea pig serum from which the antiferments have been removed by extraction with chloroform, the serum then being incubated to allow the

ferment present to split the serum protein) note that small doses increase coagulation time, the large doses tend to produce intravascular clotting. By first giving hirudin they were able to kill the animals with the toxic serum without evidence of intravascular clotting; therefore they do not believe that the intravascular clotting can be the cause of death in protein poisoning. They offer, however, no explanation of the clotting. Von Behring (18) has advanced the theory that the cause of death in anaphylaxis is the intravascular clumping of the blood platelets in the small vessels of the brain.

In the course of this work a number of rabbits were lost in a peculiar way. It was attempted to sensitize the rabbits by three injections at intervals of one to three days, one intravenous, one intraperitoneal, and one subcutaneous. The intravenous injection if preceded by one or both of the others very often caused the immediate death of the animal. The amount injected intravenously was usually 0.5 cc. of egg-white (diluted) or 1 cc. of beef serum. Often before one-third of the dose had been injected the animal would have a violent convulsion and die immediately. This never happened if the intravenous injections were given first. Gross inspection of these animals at autopsy failed to show intravascular clotting. The death was certainly not due to anaphylaxis as the term is ordinarily understood, for the fatal injection followed the first injection after an interval too short to allow sensitization to occur; and the rabbit is not sensitized as readily as are some other animals. In another group of animals that received intravenous injections of protein before sensitization could be expected to occur, death occurred several days after the intravenous injection, and at autopsy extensive intravascular clotting and pulmonary infarction were found. Various attempts were made to analyse the mechanism of this reaction, but no satisfactory explanation was reached. Lee and Vincent (19) find that the increased coagulation time in anaphylaxis is due to the clumping of the blood platelets, and their consequent withdrawal from the peripheral circulation. It might be suggested in explanation of the death of my animals that in the cases of acute death, death was due to

cerebral emboli of platelets, and that in those cases dying later the emboli of platelets formed a nucleus from which the extensive clots formed that were found at autopsy. This, however, is not in complete accord with the work of Lee and Vincent, for they found that in those cases in which the signs of anaphylaxis were only mild in degree, there was little or no delay in coagulation time, nor disturbance of platelet function, and in my cases the ordinary signs of anaphylaxis were not present. No adequate explanation of these deaths is at hand.

RESULTS

Material was examined from 23 guinea pigs, including 6 controls, with the following findings:

Hyperemia was marked in all cases dying acutely, and was most marked in the glomeruli. It was observed in other cases but was never marked.

Hemorrhage was frequently present, especially in those cases dying acutely, but was never extensive.

Degeneration, necrosis, and desquamation of tubular epithelium. This was the most striking change observed; it was seen in all cases, and was usually quite prominent. The process was most noticeable in the proximal convoluted tubules, but was also seen in the ascending limb of Henle's loop, in most cases. Occasionally some of the other tubules were involved. In the mildest cases the cytoplasm of the epithelial cells was swollen, and the margins were ragged or frayed. In the more marked cases vacuoles of varying size formed at the bases of the cells, and the nuclei were pyknotic. These vacuoles do not take the stain in the Marchi osmic acid technique. Apparently these vacuoles increased in size until the cytoplasm resembled a badly torn network, or was displaced toward the center of the tubule, giving the appearance of desquamation, and the nuclei were fragmented. This was the stage that was most commonly observed. In nearly all cases actual desquamation was observed in certain tubules, and regeneration was frequently seen. The vacuolation was confined almost entirely to those cases dying

acutely or to those killed shortly after the last injection of protein. In those killed three or more weeks after the last injection, the vacuolation was much less prominent or absent, while necrosis and desquamation were more prominent. The earlier stages of this process were observed in those controls that received injections of protein, but only in a few isolated tubules, and were more likely to be found in the loops of Henle than in the convoluted tubules, while the opposite was true of the experimental animals.

Swelling and proliferation of the capillary endothelium of the glomerular tufts were observed in 12 (70 per cent) of the cases. With two exceptions, this change occurred only in animals that had been under observation for three months or longer. The two exceptions were guinea pig 25, which had been under observation for six weeks, and had experienced four anaphylactic shocks, and guinea pig 31, which had been under observation for four weeks and had experienced five anaphylactic shocks. In a few cases there was proliferation of the capsular epithelium, and in one case fibrous thickening of the capsule was seen in many glomeruli. No glomerular lesions were seen in any of the controls.

Round celled infiltration was noted in nearly all cases, but was never marked. The areas were small and scattered diffusely over the section, but especially in the cortex, and were usually located about blood vessels. Usually these areas were quite numerous, but occasionally only a few were seen. Guinea pigs, apparently normal, may sometimes show small areas of round celled infiltration in the renal cortex (20) and such areas were found in some of the controls, but they were always much fewer in number than those found in the experimental series, not more than two or three to a section, or about one-tenth to one-fifteenth the number found in the experimental animals.

The character of the tissue lesions, their distinctly focal distribution, and the frequent occurrence of hemorrhages strongly suggest the possibility that the lesions may be dependent on vascular changes. Vascular lesions of an acute or subacute type were found in 82 per cent of the experimental animals dying

from anaphylactic shock or killed within two weeks after the last injection, and in 50 per cent of the animals killed from three to eight weeks after the last injection. These lesions occurred especially in the small vessels, and consisted in a swelling of the arterial wall, with, in some cases, very marked degeneration of the intima, and vacuolation and fissuring of both intima and media. Sometimes there was a marked proliferation of the endothelium. When this change was found in an animal it usually involved all or nearly all of the small vessels in the section. Rarely this was seen in the larger vessels. These lesions were not seen in any of the animals dying after one or two shocks, nor in normal controls.

In a few cases edema of the interstitial tissue of the papillae was seen.

Distinct scar formation was not seen in any case.

The significance of these results lies in the possibility of interpreting them as at least a partial explanation of the etiology of some cases of arteriosclerosis, and of some cases of nephritis, especially of the arteriosclerotic type. This will be discussed in more detail in a later paper.

CONCLUSIONS

Repeated anaphylactic shock induced in guinea pigs by injections of egg-white or beef serum is able to produce lesions of the kidney that are not produced by acute anaphylaxis, nor by the repeated injection of these proteins in refractory animals. These lesions consist principally of necrosis of tubular epithelium, proliferation of glomerular capillary endothelium, and swelling and degeneration of the intima and media of small vessels. Small diffusely scattered areas of round celled infiltration were observed in nearly all cases, somewhat similar to the areas observed in the controls, but usually larger, and invariably much more numerous than the spontaneous lesions. In this series the lesions noted are to be considered as subacute rather than chronic.

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POSTSCRIPT

Since this work was completed, an article has appeared by Longcope (Longcope, W. T., Journ. Exp. Med., xxii, 6, p. 793) in which he describes changes in parenchymatous organs similar to those previously reported by him (but less marked, and less frequently obtained), produced by a single large dose of foreign proteid, and he ascribes these changes to the gradual splitting of the protein within the animal's body, and the gradual liberation of the toxic substances. In applying these results to the problems of human disease it matters not whether the toxic substance is liberated slowly from a single quantity of protein, or rapidly from many quantities, the toxic substance is the same, and the results produced are the same, in character if not in degree.

FIG. 1. GUINEA PIG 32

Necrosis of tubular epithelium after four injections of egg-albumin and three of beef serum in a sensitized animal, dying two weeks after last injection.

FIG. 2. GUINEA PIG 18

Vacuolation of tubular epithelium after four injections of egg-albumin and three of beef serum, in a sensitized animal dying very soon after the last injection.

FIG. 3. GUINEA PIG 19

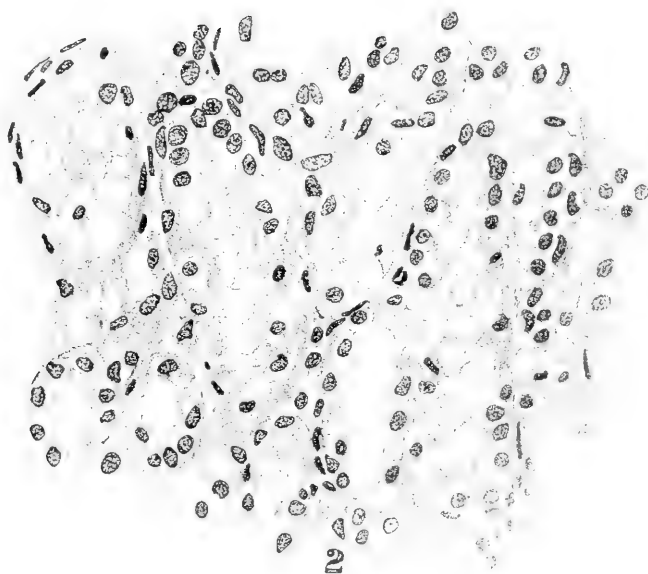
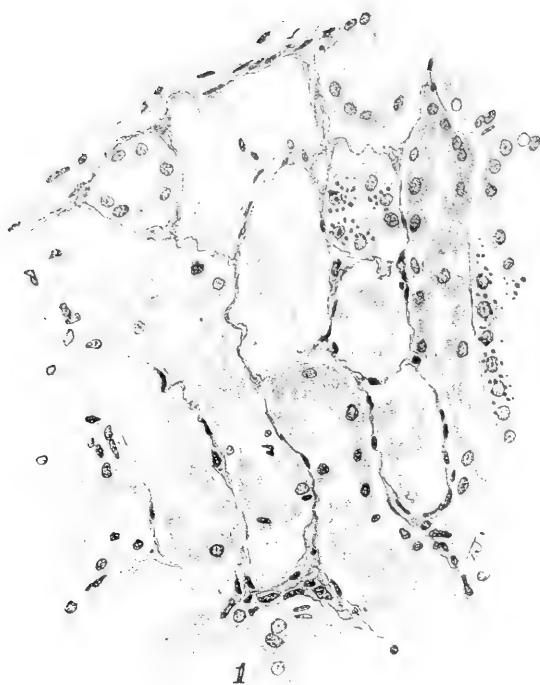
Round celled infiltration in the cortex of the kidney after seven injections of egg-albumin and three of beef serum in a sensitized animal, killed five weeks after last injection.

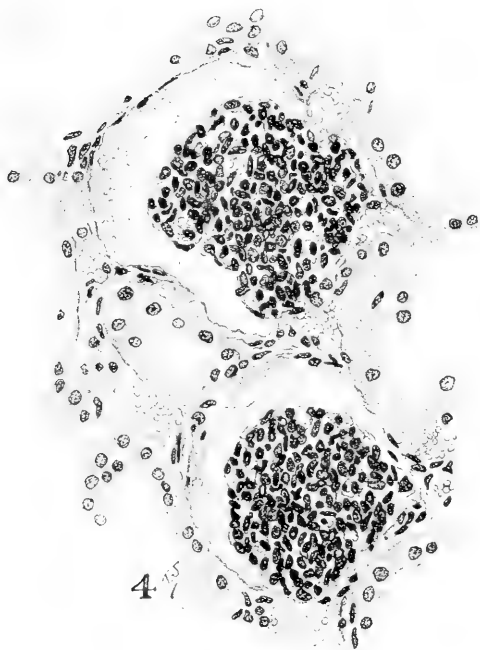
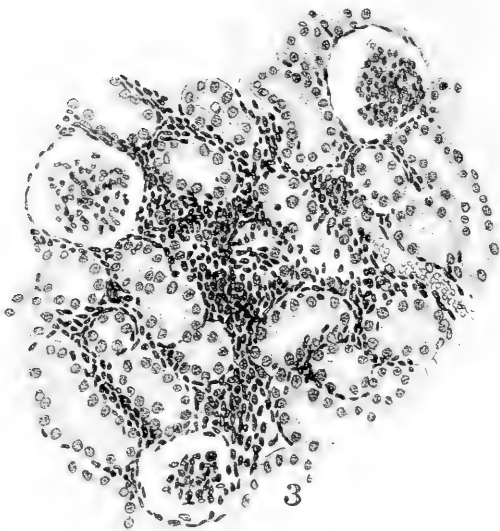
FIG. 4. GUINEA PIG 20

Proliferation of capillary endothelium of glomerular tuft of kidney, with coagulum in capsular space after six injections of egg-white and five of beef serum in a sensitized animal, killed three weeks after last injection.

FIG. 5. GUINEA PIG 24

Swelling, vacuolation, fissuring of intima and media; marked degenerative changes and proliferation of endothelium of intima, in arteries of the kidney. after six injections of beef serum in a sensitized animal; death from anaphylaxis. Hematoxylin and eosin stain. Note splitting of elastic membrane in artery A,





THE SITE AND RATE OF DESTRUCTION OF PNEUMOCOCCI FOLLOWING INTRA- PERITONEAL INJECTION

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In a recent publication concerning the physiological destruction of erythrocytes, Kyes (1) has shown that certain endothelial cells of the liver and spleen are constantly active in phagocytosing red blood corpuscles from the circulating blood stream and to the fixed-tissue phagocytes so functioning, he gives the designation "hemophages."

In a subsequent communication concerning the natural immunity of the pigeon to the pneumococcus, the same author (2) points out that under experimental conditions, the hemophages are not only phagocytic for red blood corpuscles but also for pneumococci introduced into the circulating blood stream. Indeed, so rapid and so extensive is the phagocytic destruction of injected pneumococci by the hemophages of the pigeon, that Kyes concludes that this mode of elimination of the organisms from the blood stream is largely responsible for the high resistance which the pigeon displays to pneumococcus infection. The great importance of the hemophages in this immunity appears the more probable since Kyes observed that pneumococci introduced into the body cavities of the pigeon were rapidly transported to the blood stream and were then also eliminated by the hemophages after the same manner as those organisms injected directly into the blood stream.

The present study is a further analysis of the extent to which pneumococci introduced into the peritoneal cavity are destroyed by hemophages in the liver and spleen and is also a determination of the rate of the destruction of the bacteria so introduced in comparison with those injected directly into the blood stream.

The fate of inert, insoluble particles injected into the peritoneal cavity has been investigated by Muscatello (3). Employing a suspension of insoluble carmine, this worker found that after a period of from one and one-half to two hours, the pigment granules were present in the liver and spleen, either free within the blood vessels or within leucocytes of the blood stream. In smaller numbers, he also found the carmine granules in the lung, pancreas, and the testes. Maffuci (4) conducted similar experiments, and after the same lapse of time also found the pigment particles in the liver and spleen, but in those organs only. Maffuci decided also, that the position of the granules was the same in the liver whether the injections had been made into the peritoneal cavity or the jugular vein. Buxton and Torrey (5) in an extended series of experiments with lamp-black injected into guinea pigs, further confirmed the general findings of Muscatello. In studying also the distribution of typhoid bacilli injected intraperitoneally into rabbits, Buxton and Torrey showed by cultural methods, that the bacilli very rapidly reached the blood stream and were deposited in various organs, "the liver showing the greatest power of holding up to the organism." Strouse (6) on the other hand, working with intraperitoneal injections of pneumococci into pigeons did not find a distribution of the organisms to the liver or spleen and in but two pigeons of a series of fourteen did he recover the organisms from the heart's blood.

EXPERIMENTS

With the purpose of comparing the fate of pneumococci injected intravenously with that of those introduced intraperitoneally, we injected parallel series of pigeons by these two avenues with the same amounts of one and the same suspension of pneumococci and killing the animals after the lapse of various time intervals, determined the distribution of the organisms within the various tissues by systematic microscopic examination. Recognizing the unreliability of fine quantitative distinctions based upon the direct numerical count of bacteria in tissues, we have neglected minor differences and taken into consideration only contrasts of such degree as to be beyond the limits of error of observation and of interpretation. The pneu-

mococcus culture used was one freshly isolated from a case of acute lobar pneumonia in man. This organism was grown on blood agar slants in one litre flasks, the surface of each of which was approximately equivalent to that of twenty ordinary blood agar slants in test tubes. After incubating these flask cultures for twenty-four hours at 37°C. the total growth was suspended in such a quantity of physiological salt solution that two cc. of the suspension contained approximately the same number of pneumococci that would be obtained from two and one-half blood agar slants. For a single series twenty-two pigeons were used—eleven receiving 2 cc. of the above suspension intravenously, and eleven receiving 2 cc. intraperitoneally. These birds were killed after the lapse of 10 minutes, 30 minutes, 1, 2, 3, 6, 9, 12, 18, 24, and 36 hours following injection. Immediately after killing, thin pieces of the liver and the spleen from each bird were placed for 24 hours, in Zenker's solution without acetic acid. After imbedding in paraffin, sections 3 to 4 μ in thickness were cut and fixed on slides. The sections were further prepared by the method used by Kyes (2) in his study of pneumococci injected intravenously into pigeons. Briefly the method is as follows: To differentiate the hemophages the sections are first subjected to treatment with potassium ferrocyanide and hydrochloric acid. They are then stained with acid carmine and by Gram's method. A point of considerable importance in connection with the latter is the careful decolorization in a mixture of toluol and aniline oil, approximately in the proportion of two to one. The use of alcohol after Gram's stain decolorizes the specimen too rapidly and completely. With the technic mentioned, the Gram positive pneumococci when present, stand out prominently in the distinctly differentiated blue-green hemophages.

When the tissues of a complete series of birds were fixed as outlined above, each observer stained and examined a series of slides from each pigeon and tabulated his results independently. These results were then compared and gone over again where there was any difference of opinion, so that the combined results represent observations on two complete sets of slides from the same bird, each set being prepared and examined by a different

individual. As an additional control on the tissues which failed to show pneumococci in the double staining method, at least five slides from such tissue were stained with Gram's stain alone, before it was concluded that the organisms were not present.

Discussion of Results

The results obtained in the four different series were parallel and uniform except for slight individual variations which were inconsiderable from the point of view of the demonstration in question. The protocol of the single series here reported is illustrative therefore of the total four series investigated.

Stated in general terms, the results obtained by the methods outlined showed that following intraperitoneal as well as intravenous injection, the pneumococci are phagocytosed by hemophages in the liver and spleen and furthermore that the organisms so taken up are rapidly destroyed within the containing cell. Also, that the appearance of the pneumococci in the liver and spleen is later following intraperitoneal than intravenous injection but as extensive. Before discussing the results in detail however, the approximate quantitative results obtained may be charted as follows:

TABLE 1

TIME INTERVAL	CONCENTRATION OF ORGANISMS			
	Intraperitoneal		Intravenous	
	Liver	Spleen	Liver	Spleen
10 min.	0	0	+	+
30 min.	0	0	+ ²	+ ²
1 hr.	0	0	+ ⁶	+ ⁶
2 hrs.	+	+	+ ⁸	+ ⁷
3 hrs.	+ ⁴	+ ⁴	+ ⁷	+ ⁶
6 hrs.	+ ⁸	+ ⁸	+ ²	+ ²
9 hrs.	+ ²	+ ²	+	+
12 hrs.	+	+	+	+
18 hrs.	0*	0*	+	+
24 hrs.	0*	0	0*	0*
36 hrs.	0	0	0	0

* Possibly a few remnants of digested cocci, but no organisms of definite form or staining reaction.

The results tabulated above show that tremendous numbers of pneumococci were distributed to the liver and spleen not only when introduced intravenously but also when injected intraperitoneally. Furthermore the pneumococci within these organs were located for the most part within hemophages, irrespective of the mode of injection.¹

The single point of difference in the distribution of the bacteria following the two modes of injection is to be observed in regard to the time of the localization of the organisms within the liver and spleen. Following intravenous injection of the bacteria, their partial localization in the liver and spleen is accomplished at the time of the earliest observation, namely ten minutes. With the intraperitoneal introduction on the other hand, the first evidence of such localization occurs at the two-hour observation. The same relation obtains also in regard to the period of maximum accumulation of the bacteria in these organs. Thus, the pneumococci were found in greatest numbers in the hemophages of the liver and spleen two and three hours after intravenous injection, whereas after intraperitoneal injection, the maximum bacterial content was found occurring distinctly later, namely from three to six hours after the injection.

The period of survival of the pneumococci after their accumulation within the liver and spleen cannot be determined with any great degree of accuracy. This is because it is impossible to identify absolutely the last remnants of the digested organisms and therefore to establish the exact time of the complete disappearance of bacterial remains. Our experiments do show however, that both the liver and spleen of birds injected intravenously show a much reduced content of organisms at the six hour period, a corresponding reduction appearing at the nine-hour period of birds injected intraperitoneally. At subsequent periods the number of organisms found was inconsiderable, only partially digested remnants being present after eighteen hours and even these being entirely absent at thirty-six hours. At

¹ The details as to localization will not be included here in as much as they coincide with the descriptions already given in extenso by Kyes in a communication previously referred to.

the twenty-four-hour period no intact, normally staining organisms were present, but it was impossible to determine whether or not occasional dark granules within the hemophages were end products of bacterial digestion. Without attempting therefore to establish the exact time of their complete disappearance, it may be stated that under the conditions of our experiments, the bulk of the pneumococci localized in the liver and spleen are destroyed within six hours after the first appearance of the bacteria in those organs.

CONCLUSIONS

The conclusions which may be drawn from the foregoing results are:

1. That pneumococci injected intraperitoneally into pigeons are rapidly transported to the liver and spleen and are there localized within fixed tissue phagocytes after exactly the same manner as are pneumococci introduced directly into the blood stream.

2. That, judged both from the time of the appearance of the first cocci and that of the maximum concentration of cocci in the liver and spleen, the period required for the transportation of the organisms from the peritoneal cavity to the circulating blood stream is approximately two hours.

3. That pneumococci which are localized in the liver and spleen following intraperitoneal injection are destroyed within twenty-four hours of their arrival in those organs.

This study was undertaken at the suggestion of Professor Preston Kyes and we desire to express our appreciation of his interest and assistance during the progress of the work.

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SCIENTIFIC PROCEEDINGS OF THE SOCIETY
FOR SEROLOGY AND HEMATOLOGY
NEW YORK

December 3, 1915

1. FURTHER STUDIES OF THE BIOLOGICAL METHODS FOR THE DIAGNOSIS OF TUBERCULOSIS

J. Bronfen Brenner, M. H. Kahn, J. Rockman, and Max Kahn:
There are many difficulties to be surmounted in applying successfully a biological test for the diagnosis of tuberculosis. The peculiar chemical properties of the tubercle bacillus have rendered it a difficult task to get reliable results by means of the complement deviation test, owing to the fact that the preparation of the antigen was fraught with many difficulties. Recently, however, Besredka cultivated the Koch bacillus on an entirely new medium, and he observed that the organism thus grown presented certain properties not usually inherent to the bacillus.

Thus in studying the antigenic properties of his cultures, Besredka found that they may be successfully used for the complement deviation test. Last year we made an extensive study of this subject and, although we found that the complement deviation test with Besredka's tuberculin is very useful in the majority of cases, it had certain properties, which may lead to obtaining nonspecific results.

We, therefore, decided to continue the studies of Besredka's tuberculin and, if possible, control the results of the complement deviation test by some other biological tests.

This study led us to the following conclusions:

Different samples of tuberculin of Besredka, though apparently identical in the mode of preparation, may differ in their specific values. Thus different samples were found to vary in the amount of lipins they contained. It is necessary to free each sample of tuberculin of all its lipin fraction, before using it for the complement deviation test. The lipins may be extracted by fat solvents, but the easiest method was found to be the separation of the protein fraction by precipitation. This method offers also the possibility of using a standard number of units of antigen, and so eliminates variations due to the quantitative differences in specific properties of different samples of tuberculin, without increasing the chance of obtaining lipotropic reaction.

It seems, however, that different samples of tuberculin may also vary qualitatively even after removal of lipins. This is apparently due to the existence of strain specificity in the antibody. The existence

of strain specificity in tuberculosis may explain why the results obtained by different investigators in the complement deviation test for tuberculosis vary so much.

Tuberculin of Besredka seems to give the best results in diagnosis by complement deviation test. Even though the test is positive in a certain number of clinically nontuberculous cases, the reaction seems to be specific. At least in 87 per cent of such cases, the fixation was also obtained with one or more tuberculins other than that of Besredka.

The attempt to control the serum findings by the urinary examination for urochromogen was not successful in general, and we were unable to confirm the reports of frequent occurrence of the Weisz reaction in tuberculosis. The comparison of the frequency of occurrence of the two reactions in different stages of the disease confirms our earlier suggestion that negative serum findings in the face of a positive Weisz reaction may have unfavorable prognostic significance.

2. DEMONSTRATION OF VARIOUS STAINING METHODS

F. M. Huntoon: A method of double-staining bacterial spores and bodies in a single operation. Rather heavy smears of the organism to be tested are made in the usual manner and fixed by heat; they are then covered with the staining mixture and steamed for one or two minutes, and now thoroughly washed in running tap water.

Spores appear red, the bodies of the bacteria blue.

Staining mixture: Equal parts of a 4 per cent solution of acid fuchsin in 2 per cent acetic acid and a 2 per cent solution of methylene blue (Grüblers) in 2 per cent acetic acid are mixed. The resulting precipitate is filtered off and the *filtrate* employed for staining. (Anthrax demonstrated.)

A simple method of staining capsules.

Diluent employed, 3 per cent solution of nutrose in distilled water (boiled one hour).

Staining mixture, a 0.5 to 1 per cent solution of methylviolet (saturated alcohol solution) in 2 per cent carbolic acid solution to which has been added 0.5 per cent lactic acid concentration and 1-10,000 acetic acid.

Make thin film employing diluent. Allow to dry in air cover with staining mixture for about thirty seconds. Wash in water, dry and examine. (*Streptococcus mucosus* and *Pneumococcus* demonstrated.)

A method of demonstrating capsule like envelopes on various bacteria, both flagellated and nonflagellated.

Thin smears are made from young broth cultures and allowed to dry in the air. Do not fix. Cover with a 5 per cent solution of carbolic acid to which has been added 1 per cent of a saturated alcoholic solution of any of the more diffuse dyes. Allow to act for fifteen seconds, pour off and allow to dry. Do not wash or blot. (*Bacillus Subtilis* demonstrated.)

3. THE LATE RESULTS OBTAINED IN THE ACTIVE IMMUNIZATION WITH MIXTURES OF DIPHTHERIA TOXIN-ANTITOXIN AND WITH TOXIN-ANTITOXIN COMBINED WITH DIPHTHERIA BACILLI

Wm. H. Park and Abraham Zingher: In an earlier communication before the Serological Society, Park and Zingher gave the results obtained with toxin-antitoxin in scarlet fever children at the Willard Parker Hospital. They showed at that time, that children with a natural antitoxic immunity gave a uniformly good response to these injections and developed an increase in the antitoxin content, which was quite considerable. In those who had no natural antitoxic immunity, successful results within the period of observation four weeks after the injections) were noted in only 25 to 30 per cent of cases.

The work was continued during the past one and one-half years and further attempts were made to obtain better results by modifying the mixtures used for immunization and by the addition of killed diphtheria bacilli. In the last group of 156 patients over 35 per cent showed enough antitoxin production within four weeks after the injections, to give a negative Schick reaction, and a somewhat larger proportion showed a fainter Schick reaction than in the control test before the injections.

The cases were followed up to their homes, and 82 immunized individuals were retested with the Schick reaction at intervals of from three months to one and one-half years after the injections. The results obtained were quite striking. All those who had given an early and successful response to the immunization were found to be still immune; of those who had failed to give an early response, or in whom only slight traces of antitoxin were produced, fully 80 to 85 per cent were now found immune.

This late development of antitoxin shows that active immunization with toxin-antitoxin is successful for the general protection of susceptible individuals against future exposure; the early results, however, show that immunization with toxin-antitoxin cannot be depended upon for the immediate protection of exposed individuals.

4. THE IMMUNE MECHANISM OF ANAPHYLAXIS

Richard Weil: Heating precipitating antibody at 72° for one-half hour produces two important effects:

a. It destroys the property of producing precipitation with the antigenic serum.

b. It destroys the property of fixing complement in the presence of the antigenic serum.

Notwithstanding these changes it retains, though in slightly diminished degree, its property of conferring passive sensitization on normal animals.

Two conclusions are drawn: First, that precipitation does not enter into the anaphylactic reaction.

Second, that complement does not enter into the anaphylactic reaction.

The latter conclusion indicates that anaphylatoxin plays no part in the mechanism of anaphylaxis, since the antigen-antibody complex never gives rise to anaphylatoxin in the test tube except in the presence of complement. If this inference is correct, the chemical theory of anaphylaxis is deprived of support, leaving the physical theory to account for the phenomenon.

The immune serum used for these experiments was that of a rabbit versus crystalline egg albumen. The hemolytic system consisted of ox erythrocytes and rabbit hemolysin. It is not known whether identical results would be given by other combinations.

FURTHER EXPERIENCES WITH THE ISOLATED ORGAN LIPOIDS AS "ANTIGEN" IN THE WASSERMANN TEST

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Our previous publications (1) upon the Wassermann reaction have dealt chiefly with the technique of the test. We have called attention to two important sources of error that attach to the use of the isolated organ lipoids (Noguchi) as "antigen." These were: first, the non-specific or "pseudo-reaction" that is sometimes obtained with the originally prescribed quantity of "antigen" when the serum to be tested has not been heated; and secondly, the prezone phenomenon of wanting or weak reaction, which sometimes occurs with positively reacting sera where the larger quantities of the lipid emulsion are used.

In a subsequent larger series of examinations we have not only confirmed those observations, but, through our association with Dr. E. L. Keyes and with the generous assistance of Dr. D. W. MacKenzie, Dr. B. S. Barringer and Dr. J. D. Kernan, Jr., we have been able, also, to reach a definite conclusion as to the relative clinical efficiency of our technical method in the diagnosis of syphilis.

The technique employed was not essentially changed from that described in our former publications. The serum to be examined was used undiluted in a quantity of 0.02 cc., or it was diluted 1 to 10 and the diluted serum was used in a quantity of 0.2 cc. The sera were examined usually in the unheated condition.

The guinea pig's serum was used in a 1 to 10 dilution in quantity of 0.1 cc.

We have found that the process of isolating the lipoids from the heart muscle can be considerably shortened by the following method: the fresh muscular tissue of the ox's heart was very finely ground in a meat chopper and mixed with 6 volumes of 95 per cent alcohol. After standing for twenty hours or longer, during which time it was occasionally shaken, the mixture was filtered and the filtrate was placed in a large porcelain evaporating dish before a large electric fan. In a few hours the greater part of the alcohol had been driven off, as was indicated by the disappearance from the fluid of the odor of alcohol, and practically all of the lipoids had been deposited on the bottom of the dish. The fluid was then poured off and the layer of lipoids adhering to the bottom of the dish was dried by being held before the electric fan. The sticky residue was taken up in a little ether and the ether-soluble portion was separated by centrifugation and decantation. The lipoids were precipitated from the ethereal solution with six volumes of acetone and the precipitate was dissolved in water-free ether and reprecipitated with acetone. After one or two further reprecipitations out of water-free ether the waxy precipitate was freed from acetone as far as possible by warming and with the use of a vacuum pump and it was then shaken with 10 to 15 volumes of absolute methyl alcohol in which a considerable portion of the precipitate was insoluble and adhered to the walls of the flask. In a few minutes the alcoholic solution could be decanted and the concentration of the lipoids in it was then determined by weighing the residue obtained by evaporating 0.5 cc. to dryness on a water-bath. On the basis of this determination the solution was diluted with absolute methyl alcohol to a calculated concentration of 2 per cent and this solution was kept in the ice-box. 0.4 cc. of a 1 to 10 dilution of this preparation may sometimes be anticomplementary in the ice-box control (18 to 24 hours at ice-box temperature) but that amount should not be anticomplementary after one hour at 37°C. The lipoids were used in a 0.2 per cent emulsion that was prepared by blowing one volume of the 2 per cent methyl alcoholic solution from a pipette rapidly into nine volumes of physiological saline solution. This emulsion was regularly

used in six different quantities with each serum: namely, 0.2, 0.1, 0.05, 0.02, 0.01 and 0.001 cc.

A constant degree of sensitization of the sheep's blood corpuscles that were used as indicator of the reaction was conveniently secured by the use, during the greater part of the study, of the same rabbit's anti-sheep's corpuscle serum, which maintained, for about a year and a half, without special care, a titer of 1 to 5000. Usually 0.25 cc. of a 1 per cent¹ suspension, later 0.1 cc. of a 2.5 per cent suspension of the sensitized corpuscles has been used.

PSEUDO-REACTION

In 32 cases, more or less (usually complete) complement-fixation was obtained only with 0.2 cc. of the 0.2 per cent lipid emulsion. Twenty-two of these were of treated syphilis without symptoms, 2 were of untreated syphilis with lesions and 5 were individuals giving no history of syphilis and exhibiting no symptoms of that disease. One of this last group was a case of malaria examined during the paroxysm. In 6 cases there was complete fixation with 0.2 cc. and partial fixation with 0.1 cc. of the lipid emulsion. Four of these were of treated syphilis without symptoms; one was of untreated tertiary syphilis with lesions; and one was without history or symptom of syphilis. On one occasion in preparing the lipid emulsion with a preparation that was nearly a year old, instead of blowing the methyl alcoholic solution of the lipoids rapidly into the saline solution, we mixed the two fluids more slowly. The resulting emulsion was milky and nearly opaque, and in the entire series of tests with this emulsion there was considerable complement fixation with 0.2 and 0.1 cc.; but 0.4 cc. of this emulsion was of itself anticomplementary. The tests were therefore repeated with an emulsion of the same lipid solution prepared as usual by blowing the required amount rapidly into the salt solution. With this emulsion, which was opalescent but not opaque, many of the sera now gave no complement fixation. 0.4 cc. of the emulsion was not anticomplementary. With a properly prepared lipid emul-

¹ Corpuscular sediment was used as the basis of calculation.

sion we have met with four instances of complete fixation with 0.1 cc. of the emulsion in individuals denying leutic infection and not exhibiting lesions of syphilis. In none of these instances was the question as to the extent of the zone of pseudo-reaction involved, since in each case fixation was obtained also with much smaller amounts of the lipoid emulsion.

The zone of pseudo-reaction, therefore, includes in our experience 0.2 cc. and a partial inhibition with 0.1 cc. of a 0.2 per cent emulsion of the lipoids of the ox's heart. If, therefore, the unit of sensitized sheep's blood is completely dissolved by a mixture of the unit of guinea pig's serum and 0.4 cc. of the lipoid emulsion after that mixture has stood for one hour at 37°C., then complete inhibition produced with the unheated human serum when combined with 0.1 cc. of the lipoid emulsion and the unit of guinea pig's serum can be accepted as a fixation of diagnostic significance. In view of the fact that most of the sera that react only within the narrow zone of pseudo-reaction are derived from individuals giving a clear syphilitic history it seems correct to designate such reactions as "questionable."

PREZONE PHENOMENON

This phenomenon has been encountered forty-four times in 203 positively reacting sera, sometimes after incubation at 37°C., sometimes after incubation at ice-box temperature. The sera were not heated for any of these examinations. It is not to be inferred that under the original plan of examination, i.e., with only one quantity of "antigen," the reaction would have been missed in all of the forty-four observed instances of the prezone phenomenon, even if the examinations had been made at only one incubation temperature. For example, the reaction in cases 600 and 929 (Table I) would have been missed at 37°C. but at that temperature a positive reaction would have been obtained in cases 431, 498, 382 and 564.

However, in 16 cases or about 8 per cent of all the positively reacting sera there was no reaction at either incubation temperature with the two larger quantities of the lipoid emulsion. Case

no. 600 in Table I illustrates this group. It is evident, therefore, that when the isolated organ lipoids are employed in the performance of the Wassermann test the use of only one quantity of "antigen" as originally prescribed exposes the test to a serious source of error.

A study of table I reveals two zones of reaction the limits of which are from 0.2 to 0.02 cc.—zone A—and from 0.05 to about

TABLE I
Illustrations of the prezone phenomenon

IN EACH TUBE 0.02 CC. OF THE PATIENT'S SERUM AND 0.1 CC. OF 10 PER CENT GUINEA PIG'S SERUM								
	CUBIC CENTIMETER OF 0.2 PER CENT LIPOID EMULSION							
	0.2	0.1	0.05	0.02	0.01	0.005	0.002	0.001
431 { 37°.....	0	0	0	0	0	0	0	0
7°.....	H	H	H	0	0	0	0	0
498 { 37°.....	0	0	0	0	0	0	0	0
7°.....	H	H	H	h	tr.	0	0	0
600 { 37°.....	H	H	H	H	H	H	H	H
7°.....	H	H	H	0	0	0	0	0
929 { 37°.....	H	H	H	H	H	H	H	H
7°.....	0	0	H	0	0	0	0	0
382 { 37°.....	0	0	0	h	H	H	H	H
7°.....	H	H	H	h	0	0	0	0
564 { 37°.....	0	0	H	H	H	H	H	H
7°.....	H	H	h	0	tr.	tr.	tr.	tr.

H = Complete hemolysis.

h = partial hemolysis.

tr. = trace of hemolysis.

0 = no hemolysis.

0.0002² cc.—zone B. The two zones, therefore, overlap each other somewhat. At 37°C. sera 564 and 382 react only within zone A, whereas at ice-box temperature (7°C.) these sera react only within zone B. In cases 431 and 498 the reaction occurs at 37°C in both zones and in case 929 at ice-box temperature both reaction-zones are represented though separated by a narrow

² The smallest quantity of the lipid emulsion used in the entire series of tests was 0.001 cc., but in a former publication we have shown that the reaction can often be produced with 0.0002 cc.

zone of wanting reaction that is in effect a prezone to reaction-zone B. In case 929 as in many other instances of peculiar reaction that are to be described the whole test was repeated and an identical result was obtained.

Polak Daniels in some experiments, which we were able to confirm, showed that after the saturation of some positively reacting sera with one "antigen" preparation it is possible to obtain further fixation of complement by the addition of another "antigen" preparation. To explain this phenomenon Polak Daniels assumed the existence in luetic sera of two reacting bodies, which must be combined respectively with two different antigenic substances in order that complement fixation may be produced. We concurred in this assumption and were at first inclined to apply it in explaining the two reacting zones demonstrated in table 1 in the use of the isolated organ lipoids. This explanation was supported by the following observations:

1. As we stated in our previous publications, the heating of sera that, in the unheated condition, react in both zones often results in the disappearance of the reaction in the upper zone A. This phenomenon is readily explained by assuming a destruction of the reacting power of one of two reacting bodies in the serum.

2. After specific treatment of an individual whose serum had reacted in both zones the reaction often disappears in zone A but remains strong in zone B. The explanation offered above can be applied here also.

We undertook to test this explanation by saturating a serum that reacted in both zones, according to the procedure of Polak Daniels with successive small quantities (0.002 cc. at each addition) of the lipid emulsion, that is, with the hypothetical "antigen b." Four additions at one hour intervals of 0.002 cc. of the lipid emulsion and of 0.05 cc. of a 20 per cent dilution of guinea pig's serum, all of the incubations having been made at 37°C. were followed each time with complete complement fixation. After the fifth identical addition partial hemolysis occurred, indicating that saturation with the above mentioned quantity or "antigen b" of the lipid emulsion was nearly complete. In the parallel identical series, therefore, instead of making the fifth addition the same as the preceding four we added

the emulsion of the lipoids in a quantity of 0.1 cc., that is in an amount presumed to contain a fixing quantity of the hypothetical "antigen a." But with this larger addition, also, the fifth portion of complement was only partially fixed. By this method, therefore, the demonstration of a second reacting substance in the patient's serum failed.

A similar test was carried out with a serum that reacted like serum no. 431, Table I, with which the conditions were more

TABLE II
Gradual saturation of the Wassermann reacting body

ORIGINAL MIXTURE	FIRST ADDITION	SECOND ADDITION	RESULT
S + A 0.005 + C. 7° C. 3 hrs.	Blood		No hemolysis
	A 0.005 + C. 7° C. 18 hrs.	Blood	No hemolysis
	A 0.1 + C. 37° C. 1½ hrs.	Blood	No hemolysis
	A 0.005 + C. 7° C. 18 hrs.	A 0.005 + C. 7° C. 5 hrs.	Blood Very strong hemolysis
		A 0.1 + C. 37° C. 1½ hrs.	Blood Nearly complete hemolysis
		A 0.2 + C. 37° C. 1½ hrs.	Blood Nearly complete hemolysis
		S + A 0.2 + C. 37° C. 1½ hrs.	Blood No hemolysis
		S + A 0.1 + C. 37° C. 1½ hrs.	Blood No hemolysis

S = 0.2 cc. of the patient's serum diluted 1-10.

A = 0.2 per cent emulsion of the lipoids of the ox's heart.

C = 0.05 cc. of a 20 per cent dilution of guinea-pig's serum.

Blood = 0.1 cc. of a 5 per cent suspension of sensitized sheep's blood corpuscles.

favorable to the experiment inasmuch as no reaction took place at ice-box temperature in zone A. The saturation was conducted at ice-box temperature and was nearly complete after the third addition of the smaller amount of the lipid emulsion. Instead of the third addition of the smaller quantity of the lipid emulsion large quantities of the emulsion were substituted in the parallel series and the incubation was then carried out at 37°C. Under these conditions, also, a further fixation failed to take place. The protocol of the second test is given in Table II.

We were obliged, therefore, to abandon the assumption of distinct pairs of reacting substances in sera and lipoid emulsion and resort to the hypothesis of a single reacting body in the sera, which may be influenced in its reacting qualities by the physical or chemical condition of the medium in which it exists or in which it is placed by the addition of the "antigen" preparations and guinea pig's serum. Recent experiments by E. Romlinger (2) point to the possibility that the Wassermann reaction depends, not upon the appearance, in the blood, of a specific reacting substance—"reagin"—but upon a change in the chemical or physical condition of the fluid medium of the blood whereby reaction is made possible between the "antigenic" substances and some *normal* constituent of the blood.

INFLUENCE OF THE COMPLEMENT AND NATURAL AMBOCEPTOR OF THE HUMAN SERA

In over 500 examinations we have made rough determinations of the complementary activity of the patients' sera by incubating 0.2 cc. and 0.1 cc. of the diluted sera with the unit of sensitized sheep's corpuscles. The few sera that were tested below 0.1 cc. were found to be only slightly hemolytic in quantities less than that amount. 0.1 cc. often produced complete hemolysis and with many such sera the Wassermann reaction was strongly positive. In some instances, on the other hand, even 0.2 cc. of the serum failed to dissolve the corpuscle unit, yet the Wassermann reaction with a number of such sera was negative. We have not been able, in any case of syphilis with lesions in which, with our technique, the Wassermann reaction was negative, to refer the result to an excess of human complement. In the same series of examinations a rough determination of the amount of natural sheep's corpuscle sensitizing substance in the sera has been made. In a number of instances in which a positive Wassermann reaction was obtained $\frac{1}{40}$ cc. of the patient's diluted serum was found to contain at least one unit of natural amboceptor. On the other hand, in a number of non-luetic cases and in four cases of tertiary syphilis with lesions

in which the Wassermann reaction was negative, not even 0.2 cc. of the patients' sera contained a single unit of natural amboceptor. In the one case of primary syphilis and the one case of secondary syphilis that gave a negative Wassermann reaction the unit of natural amboceptor was found to be 0.1 and $\frac{1}{40}$ cc. respectively. In the former case the reaction became positive after one week at which time the sensitizing unit of the serum was still 0.1 cc. In the latter case the reaction became positive after two weeks at which time $\frac{1}{20}$ cc. of the patient's serum was found to contain the unit of amboceptor.

TABLE III.

Illustrating the absence of any influence of the natural amboceptor on the Wassermann reaction when the isolated lipoids are used as antigen

CASE NO.		CUBIC CENTIMETER OF LIPOID EMULSION						UNIT OF NATURAL AMBOCEPTOR
		0.2	0.1	0.5	0.02	0.01	0.001	
264	Sensitized blood.....	0	0	0	0	0	H	0.005 cc.
	Unsensitized blood.....	0	0	0	0	0	H	
266	Sensitized blood.....	0	0	0	H	H	H	0.0025 cc. or less
	Unsensitized blood.....	0	0	0	H	H	H	

0 = No hemolysis.

H = Complete hemolysis.

With two positively reacting sera showing a high natural amboceptor content we have carried out parallel tests, using in one series sensitized blood corpuscles and in the other series unsensitized blood corpuscles. The results of these tests are shown in Table III.

It is seen that in both instances the minimal fixing quantity of the lipid emulsion was found to be the same with the sensitized blood as with the unsensitized blood.

The results of our study of the natural amboceptor and complement in the human sera in their relation to the Wassermann test seem to indicate that under our technical procedure these substances do not ordinarily influence the outcome of the test.

PERFORMANCE OF THE WASSERMANN TEST WITHOUT THE ADDITION
OF GUINEA-PIG'S SERUM

In a series of nearly 100 cases we have attempted to carry out the test without the addition of guinea-pig's serum by depending upon the complement present in the human sera. For these tests the same quantities of the lipoid emulsion were employed and the same quantity of the sensitized blood corpuscles was used as indicator. We thought that by increasing the sensitization of the corpuscles to such a degree that 0.05 cc. of the diluted human serum would usually completely dissolve the unit of sensitized corpuscles and by doubling the amount of the patient's serum used for the test, that is, using 0.4 cc. instead of 0.2 cc., we could always secure sufficient complementary action without danger of excess. In the great majority of cases our expectations were realized and the results of the tests were practically the same as those obtained in the same cases with the use of the guinea pig's serum. In two cases, however, the complementary function of the freshly obtained patient's serum was very low (0.2 cc. produced only slight hemolysis and partial hemolysis respectively); in a third case it had practically disappeared within eighteen hours, so that the result of the test after ice-box incubation over night could not be accepted; and in a fourth case—one of syphilis with lesions—the complement content was so high that the reaction with 0.4 cc. of the patient's diluted serum was negative, whereas with 0.2 cc. with and without guinea-pig's serum the result was strongly positive.

In a few instances of non-syphilitic diseases that were examined without the addition of guinea-pig's serum, complete complement fixation occurred in a single tube of the series, all of the other mixtures of the series undergoing rapid hemolysis. On repeating these tests the fixation previously observed failed to take place. Examined with the addition of guinea-pig's serum these sera were negative.

These experiences make the elimination of guinea-pig's serum from the test appear unsafe as well as impracticable.

DELAYED HEMOLYSIS WITH THE SMALLER QUANTITIES OF
"ANTIGEN"

This phenomenon was first observed in a case of chancre that was examined four weeks after the appearance of the ulcer. The result of that test and of a second test made one week later is shown in Table IV. On each occasion the tests were repeated and identical results were obtained.

In applying our method of examination to the serum of a normal individual, since there is in each of the six tubes of the series the same quantity of the patient's serum and of guinea-pig's serum, the order in which hemolysis becomes complete should

TABLE IV
Specific delayed-hemolysis in a case of chancre

IN EACH TUBE 0.02 CC. OF THE PATIENT'S SERUM AND 0.01 CC. OF GUINEA-PIG'S SERUM						
	CUBIC CENTIMETER OF 2 PER CENT LIPOIDS					
	0.2	0.1	0.05	0.02	0.01	0.001
August 28 {	0	H	H	H	H	H
	H	H	H	H	H	H
September 4 {	6 min.	15 min.	30 min.	15 min.	6 min.	4 min.
	0	H	H	H	H	H
September 4 {	0	h	0	H	H	H
	0	h	0	H	H	H

H = Complete hemolysis.

0 = No hemolysis.

be from the tube containing the smallest quantity of the lipid emulsion up to the tube containing the largest quantity, because the lipoids of themselves exert a directly anticomplementary influence. Actually, however, unless there is a pseudo-reaction, hemolysis occurs practically simultaneously in all of the tubes of the series, the control of 0.4 cc. of the patient's diluted serum with guinea-pig's serum and without "antigen" usually preceding.

With the serum under discussion (Table IV) hemolysis was complete in the tube containing the largest amount of the lipoids in six minutes whereas it required thirty minutes for hemolysis to become complete where only one-fourth of that amount of the lipoids was used. This behaviour of the serum seemed to

show that after the incubation there was less available complement where 0.05 cc. of the "antigen" had been used than where 0.2 cc. of the "antigen" had been used, and indicated, therefore, a "specific" partial fixation of complement with 0.05 cc. of the "antigen." There was, in effect, a narrow zone of weak reaction with a prezone of weaker or wanting reaction. This interpretation of the phenomenon was borne out in an examination that was made one week later when complete fixation was obtained with 0.05 cc., the prezone persisting. The disease may be assumed to have progressed during the intervening time, since treatment had not yet been instituted.

TABLE V
Specific delayed-hemolysis in a case of spinal syphilis

IN EACH TUBE 0.02 CC. OF THE PATIENT'S SERUM AND 0.01 CC. OF GUINEA-PIG'S SERUM						
	CUBIC CENTIMETER OF 2 PER CENT LIPOIDS					
	0.2	0.1	0.05	0.02	0.01	0.001
December 14 { 37° C.. 7° C..	H	H	H	H	H	H
	H	H	H	H	H	H
	6 min.	8 min.	10 min.	25 min.	25 min.	12 min.
December 21 { 37° C.. 7° C..	H	H	H	H	H	H
	H	H	H	H	H	H
	5 min.	6 min.	12 min.	12 min.	12 min.	5 min.

H = Complete hemolysis.

h = Partial hemolysis.

0 = No hemolysis.

Specific delayed-hemolysis was again observed in a case exhibiting symptoms of spinal syphilis. The individual had been treated with salvarsan and mercury about a year previously on account of gummata. The second test was made one week after the first one, the intravenous administration of neo-salvarsan having been made on the day of the first test (Table V).

Hemolysis delayed for fifteen minutes or less where the smaller amounts of the lipid emulsion were used has been observed in seven instances. Two were after incubation at 7°C. with sera that reacted positively at 37°C.; one was in a case of rachitis without luetic history and four were in cases of doubtful clinical diagnosis.

It is evident from these experiences that while delayed hemolysis with the smaller quantities of the isolated lipoids is often a sign of a specific reaction referable to some syphilitic process in the individual, it cannot be accepted as positive evidence of such a process. If it persists in a second test carried out with a fresh specimen of the patient's blood it may be looked upon as a suspicious sign and if a clear history or lesion of syphilis is present it can then be considered as a positive reaction.

EFFECTS OF HEATING UPON THE REACTING QUALITIES OF THE HUMAN SERA

Based in part on experiments by Dr. Barbara Hunt

Our previously published experiences indicated that heating of positively reacting sera often caused the appearance of the prezone phenomenon with sera that, in the unheated condition, reacted without a prezone. Our subsequent experience has confirmed this observation.

Dr. Barbara Hunt, in this laboratory, made comparative tests with the isolated organ lipoids upon heated and unheated sera. The results of her experiments have not yet been published, but through the courtesy of Dr. Hunt we are permitted to incorporate some of them in this publication. It was found that if the tests are made immediately after the sera have been heated some sera that react even strongly in the unheated condition may seem to have lost completely their power of reaction, whereas other positive sera under similar circumstances retain their reacting power. Furthermore, it was observed that in a few instances the minimal fixing quantity of the lipoid emulsion is smaller with the sera heated for one-half hour at 50°C. than it is with the unheated sera. These effects of heating are illustrated in Table VI, with the sera of cases 350 and 344.

Each of the sera was tested in two ways; first, the minimal fixing quantity of the lipoid emulsion was determined with an equal fixed quantity of the sera, which were examined unheated, heated for one-half hour at 50°C. and heated for one-half hour at 56°C.; secondly, the minimal fixing quantity of the sera,

TABLE VI
Showing the influence of heating upon the reacting power of two luetic sera.

	IN EACH TUBE 0.2 CC. 10 PER CENT PATIENT'S SERUM AND 0.1 CC. 10 PER CENT GUINEA-PIG'S SERUM										IN EACH TUBE 0.05 CC. LIPOID EMULSION AND 0.1 CC. 10 PER CENT GUINEA-PIG'S SERUM						
	Cubic centimeter of 0.2 per cent lipid emulsion										Cubic centimeter of 10 per cent Serum 350						
	0.2	0.1	0.05	0.02	0.01	0.005	0.002	0.001	0.0005		0.2	0.15	0.1	0.08	0.06	0.04	
SERUM 350																	
	0	0	0	0	0	+++	+++	+++	+++	0	0	0	0	0	0	0	
	0	0	0	+++	+++	+++	+++	+++	+++	0	0	0	0	+++	+++	+++	
	0	0	0	+++	+++	+++	+++	+++	+++	0	+++	+++	+++	+++	+++	+++	
SERUM 344										10 per cent Serum 344							
	0	0	0	0	0	0	0	+++	+++	0	0	0	0	0	0	0	
	++	+	0	0	0	0	0	+	+++	0	+	+++	+++	+++	+++	+++	
	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	

++++ = Complete hemolysis.

+++ = Strong hemolysis.

++ = Partial hemolysis.

+ = Slight hemolysis.

0 = No hemolysis.

These examinations were made immediately after the sera had been heated, the incubation for fixation being for one hour at 37°C.

unheated and heated at 50°C. and at 56°C. respectively, was determined with a fixed amount—0.05 cc.—of the lipid emulsion. The tests were performed simultaneously on the day on which the sera were heated, the incubation for fixation being at 37°C. for one hour.

With serum 350 the minimal fixing quantity of the lipid emulsion was greater with the heated serum than with the unheated serum, but was identical with the serum heated at 50°C. and the serum heated at 56°C. With serum 344 there was no fixation obtained after it had been heated for one-half hour at 56°C., although with the same serum heated at 50°C. the minimal fixing amount of the lipid emulsion appears to have been somewhat less than it was with the unheated serum. From this result with serum 344 we do not assume that the heating at 50°C. increased the reacting power of the serum; it is possible that the effect is produced through the destruction of the human complement and the development of anticomplementary substances during the heating of the serum. That the reacting power of the serum is actually diminished by the heating would seem to be indicated by the fact (shown in the right-hand column) that a much larger amount of the serum heated at 50°C. is required to produce fixation than of the unheated serum; but the more probable explanation of this result is that, with the smaller amounts of the heated serum, the quantity of the lipid emulsion used fell within the prezone of wanting reaction.

In a single instance, however, there seemed to be a real increase in the reacting power of a weakly positive serum as an effect of heating the serum at 50°C. for one-half hour. The result of this test is shown in Table VII. Even in this instance, however, the considerable extension of the zone of reaction need not have been due to a change in the reacting substance of the serum, for it may have been caused by some physical or chemical change in the medium in which it was suspended.

The complete failure of reaction in serum 344 when examined immediately after it had been heated for one-half hour at 56°C. is not to be interpreted as a permanent destruction of the reacting power of the serum, for in three other instances in which

TABLE VII

Showing extension of the zone of reaction as a result of heating

IN EACH TUBE 0.02 CC. OF THE PATIENT'S SERUM AND 0.01 CC. OF GUINEA-PIG'S SERUM						
	CUBIC CENTIMETER OF 2 PER CENT LIPOIDS					
	0.2	0.1	0.05	0.02	0.01	0.001
Serum 270, unheated.	+++	++	+	++++	++++	++++
Serum 270, 50° C. one-half hour.....	++++	++++	+	0	0	0

Incubation temperature for fixation, 7° C. for both tests.

++++ = Complete hemolysis.

+++ = Strong hemolysis.

++ = Partial hemolysis.

+ = Slight hemolysis.

0 = No hemolysis.

TABLE VIII

Showing temporary loss of reacting power as a result of heating

IN EACH TUBE 0.02 CC. OF THE PATIENT'S SERUM AND 0.01 CC. OF GUINEA-PIG'S SERUM						
	CUBIC CENTIMETER OF 0.2 PER CENT LIPOIDS					
	0.2	0.1	0.05	0.02	0.01	0.001
Serum 430, unheated.	0	0	0	0	++	++++
Serum 430, 55° C. immediately.....	++++	++++	++++	++++	++++	++++
Serum 430, 55° C. after 24 hours.....	0	0	0	0	0	++++
Serum 444, unheated.	0	0	0	0	0	—
Serum 444, 55° C. immediately.....	++++	++++	++++	++++	++++	++++
Serum 444, 55° C. after 24 hours.....	++++	++++	++++	++	++	++++
Serum 456, unheated.	0	0	0	0	++++	++++
Serum 456, 55° C., immediately.....	++++	++++	++++	++++	++++	++++
Serum 456, 55° C., after 24 hours.....	0	0	0	++	++++	++++

++++ = Complete hemolysis.

+++ = Strong hemolysis.

++ = Partial hemolysis.

0 = No hemolysis.

positive sera were completely negative when examined immediately after they were heated, these sera recovered the power of reacting upon standing for twenty-four hours in 10 per cent dilution in the ice chest. The recovery was complete in one case—430—nearly complete in another case—456—and slight in a third case—444 (see Table VIII).

The peculiar influence of heating upon the reacting quality of serum no. 498 merits special reference. The results of the test with the unheated serum and with the serum twenty-four hours

TABLE IX

Showing a loss of power to react at ice-box temperature caused by heating the serum, the power to react at 37° C. being retained.

IN EACH TUBE 0.02 CC. OF THE PATIENT'S SERUM AND 0.01 CC. OF GUINEA-PIG'S SERUM						
		CUBIC CENTIMETER OF 2 PER CENT LIPOID				
		0.2	0.1	0.05	0.02	0.01
Incubated at 37° C.	Unheated.....	0	0	0	0	0
	Heated 53° C.	0	0	0	0	0
Incubated at 7° C.	Unheated.....	++++	++++	++++	++	+
	Heated 53° C.	++++	++++	++++	++++	++++

++++ = Complete hemolysis.

++ = Partial hemolysis.

+ = Slight hemolysis

0 = No hemolysis.

after it had been heated for one-half hour at 53°C., are shown in Table IX. Whereas the power of reacting at ice-box temperature was completely lost, the reacting power at 37°C. was apparently unaffected.

INFLUENCE OF INCUBATION TEMPERATURE UPON THE WASSERMANN REACTION AS PERFORMED WITH THE ISOLATED ORGAN LIPOIDS

In our former publication we stated that the varying influence of incubation temperature upon the Wassermann reaction that had been described by Jacobsthal and later by Guggen-

heimer under the original technique is observed also when the isolated organ lipoids are employed. In the present series 828 tests have been carried out at the two incubation temperatures—37°C. for at least one hour and 7°C. (ice-box temperature) overnight, that is, from eighteen to twenty-four hours. Of these 828 sera 584 reacted negatively at both temperatures; 203 reacted positively at both temperatures; 28 reacted negatively at 37°C. and positively at 7°C.; and 13 reacted positively at 37°C. and negatively at 7°C. Differences in the manner of reaction—due to a difference in incubation temperature—that did not affect the diagnosis have been described in the discussion of the prezone phenomenon.

CHANGES IN THE RESULT OF THE WASSERMANN TEST DUE TO STANDING OF THE SERA

We have already shown that some luetic sera, when examined immediately after having been heated for one-half hour at 53° to 56°C., may react completely negatively with the isolated organ lipoids, but if the test is repeated after the heated sera have stood for an interval of eighteen to twenty-four hours, a positive reaction will result.

In four instances of known luetic infection we have obtained, with the unheated sera, a negative reaction in the examinations that were carried out on the day on which the blood had been drawn but found the reaction positive upon examining the unheated sera again after an interval of twenty-four hours or more, at which time the sera had not become anticomplementary. The results of the tests in the four cases—no. 580, 918, 1023 and 1031—are shown in Table X. In case 663 a weak positive reaction was obtained at the first test and a strong reaction resulted from the test that was made twenty-four hours later.

It could be thought that the increased reacting power just described is due to the deterioration of the complementary power of the human sera and for this view there is some support in the observation that in one instance—no. 1031—in which the complementary power of the serum was determined, that function

had diminished 50 per cent during the interval between the two tests. This argument, however, loses force in the face of the fact that strongly positive reactions have often been obtained with freshly taken sera that showed a complementary power as strong as it was in the five cases of Table X. This fact together with our experience with the heated sera referred to at the beginning of this chapter, makes it seem more likely that the phenomenon is due to a physical or chemical change in some element of the serum other than complement.

TABLE X

Illustrating changes in the result of the Wassermann test due to standing of the sera

IN EACH TUBE 0.2 CC. OF 10 PER CENT PATIENT'S SERUM AND 0.1 CC. OF 10 PER CENT GUINEA-PIG'S SERUM

WHEN EXAMINED	CUBIC CENTIMETER OF 0.2 PER CENT LIPOID EMULSION					
	0.2	0.1	0.05	0.02	0.01	0.001
580 { Immediately....	H	H	H	H	H	H
After 24 hours..	H	H	H	0	h	H
918 { Immediately....	0	h	H	H	H	H
After 24 hours..	0	0	0	0	0	h
1023 { Immediately....	0	h	H	H	H	H
After 24 hours..	0	0	0	0	H	H
1031 { Immediately....	0	H	H	H	H	H
After 24 hours..	0	H	H	0	0	0
663 { Immediately....	H	H	H	H	H	h
After 24 hours..	0	0	0	0	0	0

H = Complete hemolysis.

h = Partial hemolysis.

0 = No hemolysis.

In some instances of treated syphilis the reaction did not become positive till the sera had stood forty-eight hours or more, but in a few other cases without specific history of lesions positive reactions were also obtained after the sera had stood for two days or longer. This experience gives additional reason for our recommendation that the sera be examined with the use of the lipid antigen within twenty-four hours after the blood has been taken.

In no case has a positively reacting serum become negative on standing.

COMPARISON OF THE RESULTS OBTAINED WITH THE ORIGINAL
WASSERMANN TECHNIQUE AND THOSE OBTAINED WITH
THE ISOLATED ORGAN LIPOIDS

The performance of the Wassermann test as it was originally prescribed in the serum diagnosis of syphilis is surrounded with many technical difficulties, chief among which may be placed the obtaining of a "good antigen." The "antigen" preparation originally prescribed was an alcoholic or carbolyzed watery extract of the liver of a syphilitic foetus. Many laboratories, however, find it difficult or even impossible to keep themselves supplied with material from this prescribed source and under such circumstances extracts of other tissues, particularly that of normal cardiac muscle, have been substituted. It is a fact of common knowledge that the extracts of different syphilitic foetal livers vary greatly as to their availability for the Wassermann test, and even among a number of usable extracts there is often one that is considered especially "good." The criterion of "goodness," here, lies in the quantitative degree of complement fixation that occurs in known cases of syphilis and in the qualitative degree of specificity, as far as this may be determined by the application of the test to diseases other than syphilis.

The difficulty of obtaining a syphilitic foetal liver for the preparation of the "antigen" seems not to be the only disadvantage that attaches to the use of the originally prescribed "antigen" preparations. An analysis of the reports from laboratories in some of the larger European cities shows that even under circumstances favorable to the securing of suitable material and in the hands of trustworthy workers, the original technique of the Wassermann test produces results that leave much to be desired.

The percentage of positive reactions obtained in cases of untreated syphilitic lesions varies widely with the different observers, and even with two sets of observers working in the same laboratory and employing presumably the same reagents.

Thus, in the primary stage of the disease positive reactions were obtained by Hoehne, in 38 per cent; Bruck and Stern, in 48 per cent; Ritz and Sachs, in 53.5 per cent; Citron and Blaschko, in 90 per cent.

In the secondary stage of the disease positive reactions were obtained by Hoehne, in 79.1 per cent; Bruck and Stern, in 79.1 per cent; Ritz and Sachs, in 98.9 per cent; Citron and Blaschko, in 98.0 per cent.

In the tertiary stage of the disease positive reactions were obtained by Hoehne, in 63.6 per cent; Bruck and Stern, in 57.4 per cent; Ritz and Sachs, in 63.7 per cent; Citron and Blaschko, in 91.0 per cent.

The great differences in these percentages of positive reactions demonstrate that under circumstances favorable to the securing of suitable "antigen" preparations as originally prescribed, some hitherto uncontrolled factor interferes with the obtaining of uniform results with the original technique by the different workers.

Furthermore, some of these workers report different experiences with those individuals that show no signs of syphilis and deny a luetic history. In this respect the reports of Hoehne (3) and of Ritz and Sachs (4) are of especial interest.

Out of 320 cases offering neither luetic history nor symptoms pointing to syphilitic infection Hoehne reports two cases giving a positive Wassermann reaction. There were some other cases (in Table III) in which "keine gesicherten Anhaltspunkte für Syphilis vorlagen" that gave a positive reaction. Three, at least, of this latter group would seem to have required particular investigation; namely the two positively reacting cases of tuberculosis and the one case of uraemia. However, we are left in ignorance of the further history of these cases, so that the diagnosis remains uncertain. The results in these five cases do not, according to Hoehne, offer any ground for doubt as to the specificity of the Wassermann reaction. Indeed, in clinically doubtful cases he accepts a positive reaction as pathognomonic evidence (5) of luetic infection.

In the report of Ritz and Sachs, also, information is lacking as to the subsequent clinical history and diagnosis in the clinically doubtful cases of "Table II;" and it is to be assumed that, in those cases, whenever the Wassermann reaction was positive the existence of syphilis was considered proven. Yet out of 980

individuals that denied having had syphilis the reaction was found to be positive in 130; i.e., in over 13 per cent; and out of 2213 individuals in whom luetic history was "questionable or lacking" the reaction was positive in 461; i.e., in over 20 per cent. Against the possibility of some of these "positive" reactions having been found in non-luetic individuals, the authors argue that, in their experience there was no disease beside syphilis in which positive reactions were especially frequent. But analysis of "Table II" seems to show that among those individuals denying a history of syphilis the positive reaction is more frequent in pregnancy (20 per cent), in tuberculosis (three cases) and tumor (three cases) than in the other conditions mentioned in the table.

It is evident, then, that relatively many more positive reactions were obtained by Ritz and Sachs than by Hoehne in individuals denying a history of syphilis; and since the former authors express no doubt as to the specificity of the "positive" reaction as obtained with their technique, it must be inferred that they look upon the additional positive reactions in this group as so much to the advantage of their method of examination.

It happens that the examinations forming the basis of the two reports that we have just discussed were carried out in the same laboratory, Hoehne's study having preceded that of Ritz and Sachs. Hoehne's percentages of positive reactions in the different stages of syphilis were relatively low. Ritz and Sachs, referring to this fact, state that in order to improve upon Hoehne's figures they have modified the original Wassermann technique, which was employed by Hoehne, first, by using, instead of *half* of the amount of extract that, by itself, was not anticomplementary, the *whole* of that amount; secondly, by "reading the test as soon as the controls permitted it." With these changes in technique a great increase in the percentage of positive reactions was obtained with sera of known luetic origin, and this is the chief support of the belief of Ritz and Sachs that as many as 13 per cent of individuals denying luetic history are infected with syphilis and are in such a condition as to exhibit a positive Wassermann reaction.

Our own experiences with the Wassermann reaction as performed with the isolated lipoids of the ox's heart and with the alcoholic extract of guinea-pig's heart muscle make it seem probable that many of the positive reactions reported by Ritz and Sachs under "Table II" were non-specific reactions.

With the isolated ox-heart lipoids employed in the manner that we have previously described we have obtained the following percentages of positive reactions in clinically recognized syphilis:

TABLE XI
Summary of the results obtained in cases of known syphilis

	TOTAL CASES	NEGATIVE		POSITIVE		PERCENTAGE OF POSITIVE RESULTS
		First test	Second test ¹	First test	Second test	
						<i>per cent</i>
Only the primary lesions present.....	35	5	1 ²	30	34	{ 1st test 85.7 2nd test 94.0
Secondary lesions present.....	42	2	1 ³	40	41	{ 1st test 95.0 2nd test 97.6
Tertiary lesions present						
(a) No treatment within six months.....	65	8		57		87.6
(b) Under treatment.....	14	9		5		35.7
(a) and (b) Taken together.	79	17		62		78.4
Latent syphilis						
(a) No treatment within six months.....	47	30		17		36.0
(b) Recently treated.....	65	52		13		20.0
(a) and (b) Taken together.	112	82		30		26.7

¹ Made *before* the appearance of the eruption.

² Became positive upon the appearance of the eruption.

³ This negative case was given salvarsan upon the finding of spirochaete and several subsequent tests resulted negatively.

On the other hand, out of 248 diseased individuals exhibiting no syphilitic lesions and from whom no history of syphilis could be obtained only 4 (less than 2 per cent) gave positive reactions.

With our method of employing the isolated ox-heart lipoids we have obtained, therefore, a relatively *low* percentage (compared with the figures of Ritz and Sachs) of positive reactions

TABLE XII

Summary of the results in individuals giving no history of syphilis and showing no symptoms of syphilis

CLINICAL DIAGNOSIS	NUMBER OF CASES	NEGATIVE	POSITIVE	PERCENT-AGE POSITIVE
Tuberculosis.....	23	23	0	
Chorea and rheumatism.....	5	5	0	
Rickets.....	6	6	0	
Non-syphilitic eruption.....	9	9	0	
Malaria.....	3	3	0	
Malignant tumor.....	39	38	1	
Jaundice.....	5	5	0	
Nephritis.....	6	6	0	
Tonsilitis.....	4	4	0	
Furunculosis.....	1	1	0	
Blastomycosis.....	1	1	0	
Pneumonia.....	2	2	0	
Eclampsia.....	1	1	0	
Chancroid.....	20	20	0	
Surgical and others.....	123	121	3	
Total cases.....	248	245	4	1.6 per cent

in individuals denying luetic history, and a relatively *high* percentage of positive reactions in clinically recognized syphilis.

Similar differences were observed in the results obtained in our own parallel tests with the isolated lipid antigen and the alcoholic extract of guinea-pigs' heart muscle in a series of 706 cases.

The extract of the guinea pigs' heart muscle was prepared by placing the finely comminuted muscle of several hearts in an amount of 95 per cent alcohol that was equal to 10 cc. to each heart and filtering off the fluid after twenty-four to forty-hours. The extract was allowed to stand at room temperature and was replaced with a fresh preparation at the end of two weeks. It was used in a 1 to 5 dilution in saline solution, the extract and saline solution being mixed slowly by layering and gentle shaking. Preliminary determination of the anti-complementary action of the extract was made immediately (on the same day) before each series of examinations. The reaction was always carried out with one-half of the smallest slightly anti-complementary quantity

of the extract and with one-quarter of that quantity. The results obtained with the larger amount of antigen are the ones considered in this comparative study. The patient's serum was heated for one-half hour at 55°C. and was used in a single amount of 0.02 cc. undiluted. The guinea pig's serum was diluted 1 to 10 and of this dilution 0.1 cc. was used in each tube. The reaction mixtures were incubated for one hour and twenty minutes for the fixation, in a bath of crushed ice and water.

In 650 of the 706 cases the results of the tests were the same with the two "antigens." In the remaining 56 cases different results were obtained as follows:

Positive with the alcoholic extract; negative with the isolated lipoids

	<i>cases</i>
No history nor symptoms of syphilis.....	27
(a) Tuberculosis.....	16
(b) Carcinoma.....	4
(c) No definite clinical diagnosis.....	5
(d) Coma.....	1
(e) Gastric trouble.....	1
Treated syphilis without symptoms.....	14
Late syphilitic lesions.....	4
Primary sore.....	2
Latent syphilis without lesions.....	1

Positive with the isolated lipoids; negative with the alcoholic extract

No history nor symptom of syphilis.....	2
(a) Carcinoma.....	1
(b) Puerperal fever.....	1
Treated syphilis without symptoms.....	1
Late syphilitic lesions.....	4
Primary sore.....	1

A further series of comparative examinations was carried out chiefly upon individuals suffering with diseases other than syphilis. The material for most of these examinations was obtained at St. Joseph's Hospital in New York City through the courtesy of Dr. J. D. Kernan, Jr. The results of the tests are shown in Table XIII.

All of the tests performed with the guinea-pig's heart extract were carried out with heated patient's serum—56°C. for one-half hour. Cases 757 to 770 inclusive were examined at the same

TABLE XIII

	FIXATION TEMPERATURE	CUBIC CENTIMETER OF ALCOHOLIC EXTRACT OF GUINEA-PIG'S HEART MUSCLE, 1-5 IN NORMAL SALINE SOLUTION						FIXATION TEMPERATURE	CUBIC CENTIMETER OF 2 PER CENT METHYL ALCOHOLIC SOLUTION OF O-HEART LIPOIDS, 1-10 IN NORMAL SALINE SOLUTION					
		0.2	0.1	0.05	0.02	0.01	0.001		0.2	0.1	0.05	0.02	0.01	0.001
Antigen control. Patients serum omitted.....	°C	0	c	c	—	—	—	°C	c	c	c	—	—	—
20 cases of treated syphilis and other diseases.....	37	c	c	c	c	c	c	37	c	c	c	c	c	c
6 cases of secondary and tertiary syphilis.....	7	0	0	0	0	0	0	7	0	0	0	0	0	0
764 Pulmonary tuberculosis.....	37	c	c	c	c	c	c	37	c	c	c	c	c	c
	7	0	0	0	0	0	c	7	st	c	c	c	c	c
765 Pulm. t. b.....	37	st	st	c	c	c	c	37	c	c	c	c	c	c
	7	0	0	0	0	m	c	7	c	c	c	c	c	c
769 Pulm. t. b.....	37	0	c	c	c	c	c	37	c	c	c	c	c	c
	7	0	0	c	c	c	c	7	c	c	c	c	c	c
797 Pulm. t. b.....	37	0	0	0	0	m	c	37	c	c	c	c	c	c
	7	c	c	c	c	c	c	7	c	c	c	c	c	c
796 Pulm. t. b.....	37	0	0	c	c	c	c	37	c	c	c	c	c	c
	7	c	c	c	c	c	c	7	c	c	c	c	c	c
757 Pulm. t. b.....	37	0	st	c	c	c	c	37	c	c	c	c	c	c
	7	0	0	c	c	c	c	7	c	c	c	c	c	c
758 Pulm. t. b.....	37	m	c	c	c	c	c	37	c	c	c	c	c	c
	7	0	0	c	c	c	c	7	c	c	c	c	c	c
759 Pulm. t. b.....	37	0	c	c	c	c	c	37	c	c	c	c	c	c
	7	0	0	m	c	c	c	7	c	c	c	c	c	c
760 Pulm. t. b.....	37	c	c	c	c	c	c	37	c	c	c	c	c	c
	7	0	c	c	c	c	c	7	c	c	c	c	c	c
761 Pulm. t. b.....	37	c	c	c	c	c	c	37	c	c	c	c	c	c
	7	0	c	c	c	c	c	7	m	c	c	c	c	c
762 Pulm. t. b.....	37	c	c	c	c	c	c	37	c	c	c	c	c	c
	7	0	c	c	c	c	c	7	c	c	c	c	c	c
766 Pulm. t. b.....	37	c	c	c	c	c	c	37	c	c	c	c	c	c
	7	0	c	c	c	c	c	7	c	c	c	c	c	c
767 Pulm. t. b.....	37	c	c	c	c	c	c	37	c	c	c	c	c	c
	7	0	c	c	c	c	c	7	c	c	c	c	c	c
768 Pulm. t. b.....	37	c	c	c	c	c	c	37	c	c	c	c	c	c
	7	0	c	c	c	c	c	7	c	c	c	c	c	c
770 Pulm. t. b.....	37	0	c	c	c	c	c	37	c	c	c	c	c	c
	7	m	c	c	c	c	c	7	c	c	c	c	c	c
798 Pulm. t. b.....	37	0	c	c	c	c	c	37	c	c	c	c	c	c
	7	c	c	c	c	c	c	7	c	c	c	c	c	c

TABLE XIII—Continued

	FIXATION TEMPERATURE	CUBIC CENTIMETER OF ALCOHOLIC EXTRACT OF GUINEA-PIG'S HEART MUSCLE, 1-5 IN NORMAL SALINE SOLUTION						FIXATION TEMPERATURE	CUBIC CENTIMETER OF 2 PER CENT METHYL ALCOHOLIC SOLUTION OF OX-HEART LIPOIDS, 1-10 IN NORMAL SALINE SOLUTION					
		0.2	0.1	0.05	0.02	0.01	0.001		0.2	0.1	0.05	0.02	0.01	0.001
	°C							°C						
799 Pulm. t. b.	37 0	} c	c	c	c	c	c	37 0	} c	c	c	c	c	c
	7 c							7 c						
815 Pulm. t. b. admits venereal disease but not lues.	37 0	} c	c	c	c	c	c	37 0	} c	c	c	c	c	c
	7 0							7 0						
816 Pulm. t. b.	37 c	} c	c	c	c	c	c	37 c	} c	c	c	c	c	c
	7 0							7 0						
842 Pulm. t. b.	37 c	} c	c	c	c	c	c	37 c	} c	c	c	c	c	c
	7 c							7 c						
843 Pulm. t. b.	37 0	} 0	m	c	c	c	c	37 0	} c	c	c	c	c	c
	7 c							7 c						
844 Pulm. t. b.	37 0	} c	c	c	c	c	c	37 0	} c	c	c	c	c	c
	7 c							7 c						
841 Pulm. t. b.	37 c	} c	c	c	c	c	c	37 c	} c	c	c	c	c	c
	7 c							7 c						
814 Pulm. t. b.	37 c	} c	c	c	c	c	c	37 c	} c	c	c	c	c	c
	7 0							7 0						
911 Malignant tumor.	37 c	} c	c	c	c	c	c	37 c	} c	c	c	c	c	c
	7 0							7 0						
802 Malignant tumor.	37 0	} c	c	c	c	c	c	37 0	} c	c	c	c	c	c
	7 c							7 c						
668 Nephritis.	37 c	} c	c	c	c	c	c	37 c	} c	c	c	c	c	c
	7 0							7 0						
727 Primary sore; eruption one week later.	37 c	} c	c	c	c	c	c	37 0	} 0	0	0	st	} c	c
	7 c							7 0						
876 Primary sore.	37 0	} 0	0	st	} st	c	c	37 0	} 0	0	0	0	0	0
	7 0							7 0						
683 Tertiary ulcer.	37 c	} c	c	c	c	c	c	37 0	} 0	0	m	} c	c	c
	7 sl							7 sl						

0 = No hemolysis.

c = Complete hemolysis.

st = Strong hemolysis.

m = Moderate hemolysis.

sl = Slight hemolysis.

time, on the day after the blood had been obtained. Case 763 was one of pulmonary tuberculosis with a clear history of syphilis but without apparent lesions of that disease. Complete fixation

was obtained in this case with 0.02 cc. of each of the "antigen" preparations.

We may summarize the results shown in Table XIII as follows:

First, since 0.1 cc. of the diluted extract of the guinea-pig's heart is the greatest quantity that, of itself, is not anticomplementary, the amount prescribed for the Wassermann test under the original technique was 0.05 cc.

Secondly, since in 20 cases of thoroughly treated syphilis without symptoms, and of other diseases, no inhibition was obtained at either incubation temperature even with 0.2 cc. of the diluted extract of the guinea-pig's heart, the specificity of the positive reactions obtained with 0.05 cc. or even with 0.1 cc. (as under the practice of Ritz and Sachs) would not ordinarily be questioned. The result of the tests in the same cases with the ox-heart lipoids was, in every instance, also, entirely negative.

Thirdly, in six cases of untreated secondary and tertiary syphilis complete fixation was obtained at both incubation temperatures with 0.001 cc. of the diluted extract of the guinea-pig's heart (i.e., 1/50 of the prescribed amount) which may be accepted as satisfactory evidence of the adequate "antigenic" power of the preparation. In all of these cases a similar result was obtained with the ox-heart lipoids.

Fourthly, out of 26 cases of tuberculosis, malignant tumor and nephritis, all denying syphilitic infection, three cases of tuberculosis reacted positively with as little as 0.02 cc. of the diluted extract of the guinea pig's heart, and nine cases of tuberculosis, one case of malignant tumor and the case of nephritis reacted positively with 0.1 cc., i.e., the quantity employed under the rule of Ritz and Sachs. All of these cases were negative with the isolated lipoids.

Fifthly, in one case of chancre, no. 727, and in one of tertiary ulceration, no. 683, the reaction was distinctly positive with the isolated lipoids but it was completely negative with even 0.1 cc. of the diluted extract of the guinea-pig's heart. In another case of primary luetic lesion—case no. 876—the reaction with the isolated lipoids was strong but with 0.05 cc. of the extract of the guinea pig's heart it was weak.

The foregoing comparative study seems to show: first, that the percentage of positive reactions obtained in the different stages of active syphilis is about the same with the two antigen preparations as employed by us; secondly, that the positive reaction produced with the isolated lipoids disappears sooner after the apparent lesions of the disease have yielded to treatment than does the reaction produced with the raw alcoholic extract; and thirdly, that the number of positive reactions obtained in individuals denying luetic infection and exhibiting no clinical symptoms of the disease is much greater with the raw extract than it is with the isolated lipoids.

The insuperable difficulties that stand in the way of determining whether all of the last mentioned group of individuals had ever become infected with syphilis make it seem inexpedient to enter into a discussion of those cases.

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IS THE HYPERLEUCOCYTOSIS FOLLOWING THE INJECTION OF TYPHOID BACILLI INTO IMMUNIZED RABBITS SPECIFIC?

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There is an extensive literature presenting experiments designed to show that the leucocytes play a more active part in ridding the organism of injected bacteria in immunized animals than in normal ones. This qualitative response on the part of the leucocytes has been very carefully studied and is specific. Gay and Claypole (1) have, however, recently described a quantitative response in the leucocytes of immunized animals which they claim is also specific. Upon injecting a normal rabbit intravenously with typhoid bacilli there results first a leucopenia and then a hyperleucocytosis; if a rabbit immunized against typhoid bacilli is similarly injected, they found likewise first a leucopenia and then a hyperleucocytosis, but the hyperleucocytosis in this case was much greater in degree than that called forth in the normal animal. Their table indicates that normal rabbits will show a maximum of about 35,000 to 60,000 leucocytes to the cubic millimeter, while the rabbits immunized against typhoid bacilli yield counts of 60,000 to 150,000. They injected a rabbit immunized against typhoid bacilli with staphylococcus aureus and observed only a slight grade of leucocytosis. They therefore concluded that the extreme hyperleucocytosis of the immunized rabbits is specific. A similar specific hyperleucocytosis was observed by them after the injection of horse serum and the red-blood cells of the sheep and guinea pig. This phenomenon which had apparently been overlooked by previous workers on the leucocytes was set forth as a new and fundamental principle in immunity, and seemed of sufficient importance to demand confirmation by other workers.

Ichikawa (2) had shown that a remarkable recovery by crisis is produced in about fifty per cent of the typhoid fever cases treated by the injection of typhoid vaccine intravenously. Somewhat later R. Kraus (3) and his co-workers in the Argentine Republic showed that a vaccine of *B. Coli* can produce the same crisis with recovery in typhoid fever as the typhoid vaccine. It occurred to us that the hyperleucocytosis described by Gay and Claypole might be connected in some way with the recovery of these typhoid fever patients after the intravenous treatment; if this were so, then R. Kraus' results would lead one to suspect that the hyperleucocytosis is not specific. This question of specificity we did not consider settled by Gay and Claypole's single experiment with the *staphylococcus aureus*, since this organism is so far removed from the typhoid group. We decided to use the *B. coli* as a control to test for specificity because of R. Kraus' favorable results in treating typhoid patients with this organism and because also it belongs to the same general group of bacteria as the typhoid bacillus, though it is immunologically distinct.

Gay and Claypole used in their experiments a typhoid culture that had been passed through two rabbits two weeks after its isolation from a human blood culture. It was grown on rabbit blood agar. One half a slant of the living culture was injected. They state, however, that "a specific response in the typhoid immune animals may be produced by the injection of various typhoid vaccines as well as by the living culture." In most of our experiments a laboratory strain of *B. typhosus* that had been carried on nutrient agar for about ten years was employed. The strain of *B. coli communis* had also been grown on artificial culture media for several years. When killed cultures were used, the suspension of bacilli was heated for 1 hour at 58° to 60°C. Gay and Claypole obtained their maximum blood counts in from eighteen to twenty-four hours after the injection, though this was preceded by a less marked crisis at about the twelfth hour. We began our counts at the eighteenth hour and continued them until the maximum was passed. This usually extended through the twenty-eighth hour, since we always wished to record two suc-

cessive counts lower than the maximum as an indication that the count was approaching normal. During this period we endeavored to make counts at one hour intervals and, as the leucocytes increased in number, even at half hour intervals. When the counts were running quite low it seemed safe occasionally to extend the interval to two hours.

The rabbit was kept in a warm room throughout the experiment, and was put into a metal box with double walls containing warm water, when blood for the counts was to be obtained. In this way free circulation in the ear vein was always assured, it being feared that impaired circulation might lead to inaccurate (too high) counts.

The rabbits included in tables 1, 2, and 3, were immunized with typhoid cultures grown on plain nutrient agar, and received such cultures also for the injection previous to the blood counts. The other rabbits were given cultures grown upon rabbit blood agar, as in the experiments of Gay and Claypole.

The rabbits shown in table 1 were both highly immunized against typhoid bacilli. Rabbit 96, which had received seven injections of large doses of *B. typhosus* during a period of two months was injected eleven days after the last immunizing injection with one-fourth of an agar slant of a killed typhoid culture. The highest count was 42,900. Seven days after this injection the rabbit was injected with one-sixteenth of a standard agar slant of killed colon bacilli. Following this injection the highest count was 43,000. Rabbit 7, which had received eight injections of large doses of *B. typhosus* during a period of two months was injected seven days after the last immunizing dose with one-fourth of a slant of a killed colon vaccine. Five days after this injection it received one-fourth of a slant of killed typhoid culture. The highest count after the colon injection was twice as much as the highest count after the typhoid injection. These two animals indicate that the hyperleucocytic response is not specific; since *B. coli* in one instance called forth just as great a leucocytosis as *B. typhosus*, and in the other case even a much higher one.

The rabbits in tables 2 and 3 were immunized by giving the injection at short intervals as recommended by Gay. Rabbit 86, typhoid immune, was injected with a heated typhoid vaccine seven days after the last immunizing injection, and seven days after this injection it received heated colon vaccine. The colon

TABLE 1

HOURS AFTER INJECTION	RABBIT 96		RABBIT 7	
	Immunized against B. Typhosus		Immunized against B. Typhosus	
	Injected with B. Typhosus after 11 days	Injected with B. Coli after 7 days	Injected with B. Coli after 7 days	Injected with B. Typhosus after 5 days
Control	13,800		8,100	9,600
18	42,300	37,000	10,400	15,400
18½	42,900	41,400		15,600
19	30,200	37,400	14,800	15,400
19½	38,800	37,400		
20	33,800		17,000	14,200
20½	33,800	32,200		
21	29,000	33,600	16,400	16,600
21½	27,600			15,400
22				13,000
22½		39,800	19,200	
23	37,600	41,000	20,400	15,200
23½		43,000		16,000
24	30,200	37,200	20,400	14,000
24½	28,000	33,100	21,000	13,800
25	28,000		32,000	
25½	23,600	39,600	30,000	16,400
26		41,900	30,100	13,200
26½	23,400	36,800	26,200	13,800
27	21,600	35,400	25,200	13,600
27½				
28		30,400		
28½				.

vaccine caused a much greater leucocytosis than the typhoid vaccine. Rabbit 85, a normal rabbit, was counted after its first injection with typhoid vaccine. Thirty-nine days after the last immunizing dose it received an injection of live typhoid vaccine. The counts were no higher in the immune than in the normal rabbit. Rabbit 93 gave an extremely high count after its first

injection with *B. coli*, but later when immunized and tested *B. typhosus* called forth a greater response than *B. coli*. Rabbit 19, typhoid immune, was injected with *B. coli* seven days after its last immunizing injection and also showed a marked leucocytosis.

TABLE 2

HOURS AFTER INJECTION	RABBIT 86		RABBIT 85	
	Immunized against <i>B. Typhosus</i>		Normal	Immunized against <i>B. Typhosus</i>
	Injected with <i>B. Typhosus</i> after 7 days	Injected with <i>B. Coli</i> after 7 days	Injected with <i>B. Typhosus</i>	Injected with <i>B. Typhosus</i> after 39 days
Control	13,800	11,500	8,600	8,600
18	23,400	39,400	20,600	17,300
18½	19,400	31,400	14,000	13,600
19	19,500	31,300	14,400	22,400
19½	19,300			19,600
20	18,700	24,800	13,200	20,000
20½	17,400			
21		26,400	13,800	15,000
21½		34,900		
22	16,800	29,900		14,000
22½			12,200	
23	18,400	24,800	14,800	
23½	18,200		15,600	12,650
24	19,200	26,800	15,500	
24½	21,000	25,300	13,200	18,300
25	18,800			22,000
25½	15,000	23,200	13,400	17,600
26			14,200	16,400
26½	18,200	32,200	14,200	14,800
27	16,800	30,600	15,200	
27½	14,200		19,600	15,400
28		26,400	13,200	14,200
28½				
29				

Since Gay used rabbit blood agar cultures in all of his experiments it was thought that our failure to get the specific extremely high hyperleucocytosis described by him might have been due to the fact that our cultures were grown on plain nutrient agar. The rabbits in table 4 and those in the experiments following were immunized and tested with cultures grown on 10 per cent

rabbit blood agar. Rabbit 82, typhoid immune, after an interval of forty-one days was injected with typhoid vaccine, and twenty days after this injection it received colon vaccine. The blood showed a slightly greater leucocytic count following the colon vaccine. Rabbit 83, colon immune, following an injection of

TABLE 3

HOURS AFTER INJECTION	RABBIT 93			RABBIT 19
	Normal	Immunized against B. Coli		Immunized against B. Typhosus
	Injected with B. Coli	Injected with B. Typhosus after 7 days	Injected with B. Coli after 7 days	Injected with B. Coli after 7 days
Control	7,200	7,800	9,800	13,800
18	22,000	17,000	17,000	19,200
18½	52,400	17,200	12,400	23,600
19	18,400	19,600		19,200
19½	19,800	17,600	19,800	
20	20,400	19,600	18,600	22,600
20½	13,600	24,000	14,400	27,600
21	12,200	22,400		24,000
21½			11,600	
22	9,700	15,600		22,600
22½			14,200	
23	13,000	24,200	10,000	26,800
23½	14,200	18,600	10,200	23,400
24	15,800			23,800
24½	15,500	25,000	11,200	21,800
25	17,800	21,000		
25½	12,800	23,000	9,400	22,100
26	12,600	25,000	11,000	
26½	14,200	24,200	13,600	22,800
27	18,200	26,800	11,200	31,100
27½	15,800	29,000	9,600	
28		26,600	10,000	29,000
28½				

live colon vaccine after an interval of forty-two days showed an extremely high leucocytosis. Forty days later when injected with typhoid it reacted similarly to a normal injected rabbit.

In none of the rabbits tested was an extremely high leucocytic maximum reached. This differed so from Gay's results that a more comprehensive experiment was planned. Since different

rabbits might presumably show wide variations in their leucocytic response to the injection of vaccine, it was decided to inject the normal rabbit and count the leucocytes, then to immunize that same animal and again study the behavior of his leucocytes

TABLE 4

HOURS AFTER INJECTION	RABBIT 83			RABBIT 82	
	Normal	Immunized against B. Coli		Immunized against B. Typhosus	
	Injected with B. Coli	Injected with B. Coli after 42 days	Injected with B. Typhosus after 40 days	Injected with B. Typhosus after 41 days	Injected with B. Coli after 20 days
Control		16,400	12,000	9,250	11,000
18	17,500		12,900	19,000	26,750
18½	18,000	36,400	20,400	17,600	24,900
19	18,000	28,800	27,200		27,150
19½	18,400		19,200	15,200	29,200
20	20,800			20,200	23,250
20½	18,800	33,200	19,300	18,200	
21	16,400			22,700	23,650
21½		67,400	25,000	18,600	21,800
22	21,200	33,800	16,600	18,750	
22½	22,600	29,200		16,650	28,950
23	19,400		23,600		23,350
23½			15,600	15,600	22,200
24	21,600	61,200	18,800	15,800	
24½	23,400		19,800		24,075
25	22,600	46,200	15,600	12,600	21,650
25½	18,300	30,000		12,600	20,650
26			14,600		20,050
26½	20,200	28,700		13,750	
27	19,250		17,800	12,750	20,700
27½	20,000	24,000	18,800	12,000	20,350
28	18,500		16,400		
28½		32,800			
29		28,700			
29½		27,400			

after the injection of vaccine. Ten normal rabbits, each weighing about 2 kilos were selected for use. The dose of typhoid vaccine administered was two thousand million per 2 kilo. The number of bacteria per cubic centimeter was determined by plating. Normal and immune rabbits received the same dose, a

larger amount having been found to be harmful to the normal rabbit. Colon vaccine was prepared in the same way, but five

TABLE 5

HOURS AFTER INJECTION	RABBIT 79			RABBIT 77		
	Normal	Immunized against B. Typhosus		Normal	Immunized against B. Typhosus	
	Injected with B. Typhosus	Injected with B. Typhosus after 40 days	Injected with B. Coli after 20 days	Injected with B. Typhosus	Injected with B. Typhosus after 41 days	Injected with B. Coli after 21 days
Control	8,800	9,450	9,000	13,400	8,400	8,800
17						40,770
17½			14,400			36,200
18	12,400		13,000	46,800	22,150	48,950
18½	9,100	16,900	12,450	35,000	20,950	45,150
19	10,500	11,350	12,800	44,600	20,200	36,575
19½	13,300	14,100	14,700	39,900	18,650	59,000
20	12,000	18,550	14,640	39,200	22,750	64,400
20½		18,000	13,100		26,825	42,000
21	9,300	22,700	19,250	37,000	23,750	
21½	11,250	18,400	13,400	30,300	20,100	36,600
22		16,000			21,000	
22½	9,400		19,200	36,800		36,200
23		16,150	19,200		17,200	
23½	9,600		15,200	36,000	19,750	26,200
24		15,150	15,050		20,200	
24½	9,000	20,200	12,600	17,500	19,350	34,850
25	9,600	18,850	15,350		19,400	33,450
25½		17,800	16,800	31,000	17,700	
26	8,800		16,600			48,750
26½	8,600	13,600	20,200	19,000	16,000	48,600
27	8,000	12,150	19,050	36,800	16,250	36,000
27½		10,800	15,200	25,300	15,600	
28	10,850	10,200		23,200		
28½	13,200			20,600		
29	12,450					
29½	14,450					
30	10,200					
30½	9,200					
31						

hundred million per 2 kilo was the amount administered. Fresh vaccine was prepared every ten days and kept on ice until used.

TABLE 6

HOURS AFTER INJECTION	RABBIT 78			RABBIT 76			
	Normal	Immunized against B. Typhosus		Normal	Immunized against B. Typhosus		
	Injected with B. Typhosus	Injected with B. Coli after 41 days	Injected with B. Typhosus after 21 days	Injected with B. Typhosus	Injected with B. Coli after 42 days	Injected with B. Typhosus after 19 days	Injected with live B. Typhosus after 9 days
Control	13,050	11,100	12,000	9,800	9,200	9,600	10,000
7			8,000	7,800			
7½			8,000		15,300	9,600	9,200
8			16,750		10,600		
8½			26,700	11,650	14,350	11,000	13,700
9			18,000	16,700	15,400	11,450	
9½			19,600	16,000	14,100	17,000	21,600
10			22,050	18,650			15,200
10½			22,650	18,600	15,400	17,600	19,000
11			28,700	15,200	13,400	16,900	20,800
11½			17,400				21,425
12				16,850	11,800	12,800	20,900
12½			19,800	18,200			26,100
13			19,000		12,700	13,700	20,200
13½			19,950	15,500			18,600
14			19,800	19,475	12,400	15,000	
14½			17,500			15,300	22,300
15			16,000	21,325	12,400	15,400	21,400
15½			16,375	17,200		13,600	20,800
16				20,025	14,500		
16½			14,200	22,300	14,700	13,250	16,000
17				18,650	18,600	15,850	
17½			15,100	15,800	16,600	15,650	16,100
18	25,400	11,400			17,350	16,000	
18½	24,800	14,200	30,750	17,100	18,200	17,000	16,100
19	21,250	13,400	19,800	16,750	15,800	16,300	
19½	14,050	20,350	18,600	21,300	16,800	14,500	12,600
20		14,400	15,700	21,000	17,500		
20½	12,200	13,200			14,750	23,250	12,200
21		13,400	20,400	16,800		20,000	
21½	16,400	13,000	18,500		11,850	15,900	16,400
22	22,750			19,100			
22½	19,750	13,400	17,600	22,000	16,000	15,650	15,000
23		26,600	21,200	23,300	10,000		
23½	22,800	20,100		21,300		15,200	19,200
24	31,250	11,800	20,400	19,900	11,450		13,750
24½	22,200	13,300	20,650			15,950	
25	17,400	14,200		17,900	15,600		22,850
25½		11,800	20,100	19,400	14,800	17,150	16,650
26	20,150	13,025	19,800	21,200		19,550	
26½	23,800			19,500	17,950	14,350	15,150
27	20,250	13,050	18,750		12,950		
27½			17,750	18,300		23,200	17,500
28	17,850	13,000		15,200			

Six of the rabbits were to be immunized against typhoid by means of three intravenous injections within a period of seven days. A control count was made of each rabbit immediately preceding each test injection. Two of the six typhoid immune

TABLE 7

HOURS AFTER INJECTION	RABBIT 75			RABBIT 74		
	Normal	Immunized against B. Typhosus		Normal	Immunized against B. Typhosus	
	Injected with B. Typhosus	Injected with B. Coli after 10 days	Injected with B. Typhosus after 10 days	Injected with B. Typhosus	Injected with B. Typhosus after 10 days	Injected with B. Coli after 12 days
Control	10,200	10,400	9,500	9,600	9,200	10,500
18	25,000	28,250	28,000	27,200	20,400	
18½	26,000	17,200	22,600	25,000	17,400	36,600
19	26,500	15,200	22,050	31,150	21,400	37,250
19½	36,500	17,800	22,400	24,800	17,400	35,600
20	27,050	17,100	35,250	26,600	18,450	39,200
20½	30,200	17,400	26,200	30,000	18,100	32,000
21	29,150	27,600		32,800	22,100	33,800
21½	28,400	17,600	25,000	40,000	25,900	
22		20,900	33,400	32,600	19,600	40,150
22½	24,800	20,450		28,800	17,200	33,000
23		20,200	25,650			44,400
23½	24,200			41,500	20,200	38,400
24		19,000	25,300	34,050	20,200	36,750
24½	21,250		23,000		20,000	
25		22,200	22,800	31,200	20,200	36,600
25½	26,800	20,250	20,650		17,500	
26	24,300	19,600	24,450	30,750	17,200	34,400
26½	24,250	18,150	24,000	29,650		34,500
27	34,400	25,200	19,900	34,600	17,400	31,200
27½	26,250	26,200	22,200	31,000	21,800	
28	26,500	22,200	23,850	39,600	18,800	28,800
28½	25,200	22,600	23,300	37,000	17,500	
29			20,350	33,100		

rabbits, nos. 79 and 77, were to receive typhoid vaccine after a long interval (about forty days), and twenty days later an injection of colon vaccine, the leucocytes being counted after both injections.

Two of the rabbits, nos. 78 and 76, were to be injected after

the same intervals of time as those above but were to receive colon vaccine first and then typhoid vaccine. Most of the animals normal and immunized showed a leucocytosis, yet the high or extreme count found by Gay in his immune rabbits was not

TABLE 8

HOURS AFTER INJECTION	RABBIT 72			RABBIT 73		
	Normal	Immunized against B. Coli		Normal	Immunized against B. Coli	
	Injected with B. Coli	Injected with B. Coli after 43 days	Injected with B. Typhosus after 19 days	Injected with B. Coli	Injected with B. Typhosus after 38 days	Injected with B. Coli after 21 days
Control	7,050	7,200	6,900	8,000	7,600	13,200
17½			10,850			31,800
18	19,250	15,450	10,600	8,200	12,325	33,600
18½	15,950	13,600			14,500	36,300
19	11,500	11,200	10,150	10,650	17,825	27,200
19½	14,400		12,600	19,000	17,800	33,300
20	13,000	15,500	13,950	11,000	17,600	35,700
20½	12,100	15,600	12,200	8,900	15,800	31,000
21		14,600		9,000		
21½	14,150	14,560	11,750		15,900	32,600
22	13,400		14,400	9,000		
22½	15,275	14,150	13,700		14,700	27,400
23	12,200	13,200	12,950	10,800	14,000	26,900
23½		12,800				
24	12,650		12,400	10,650	15,200	28,600
24½	12,300	12,500	10,650		14,950	
25		11,600		10,750	13,750	23,600
25½	12,200		10,150	11,850	13,750	21,850
26		8,800	21,975	10,400		22,000
26½	12,400		17,400	11,250	16,650	
27	11,900	9,600	14,400	12,450	17,900	21,800
27½	10,150		12,600	11,650	14,100	
28				10,600	13,000	

obtained. Four of the periods of counts were extended from the seventh to the twenty-seventh hour. Mr. Holt-Harris who had previously counted with us to control our results counted the animals used in these experiments from the twentieth to the twenty-eighth hour. Throughout this longer period of observation the

counts ran almost parallel to those made in the shorter period showing that the maximum count had not been missed and that the animals had not responded with as high a leucocytic count as Gay's.

TABLE 9

HOURS AFTER INJECTION	RABBIT 71			RABBIT 70		
	Normal	Immunized against B. Coli		Normal	Immunized against B. Coli	
	Injected with B. Coli	Injected with B. Coli after 10 days	Injected with B. Typhosus after 12 days	Injected with B. Coli	Injected with B. Typhosus after 12 days	Injected with B. Coli after 10 days
Control	6,200	6,400	6,400	13,000	9,800	11,850
17		15,000			8,900	
17½		15,000		18,500		
18	7,850	16,025	20,800	17,800	12,400	20,825
18½	7,200	17,400	19,200	17,400		16,000
19		17,500	16,900	20,600	15,050	14,050
19½	8,650	19,425	19,600	19,200	15,400	16,300
20	8,650	18,100	19,650	18,550		16,000
20½	8,800	24,100	19,000	20,200	14,850	17,425
21	8,600	24,600		19,400	12,800	15,650
21½		23,200	20,900	17,600		13,900
22	7,000	21,200	17,200		13,450	
22½			22,200	17,600		14,950
23	6,700	20,400	22,500		15,050	
23½			17,950	19,400	14,800	14,050
24	6,650	22,050		18,400	13,250	14,900
24½		23,800	21,000	18,600		14,900
25	7,250	21,200	18,450	18,000	12,900	13,400
25½	7,300	17,600	18,500	16,850	19,400	13,400
26		17,350	18,900		23,800	
26½	6,600	15,400	14,300		16,850	
27	7,000	15,000			15,000	
27½	6,800					
28						

One rabbit, no. 74, was to be injected with typhoid vaccine after an interval of ten days, then with colon vaccine after a further interval of ten days. Rabbit 75, was to be injected with colon vaccine first, then with typhoid vaccine, both injections to be made ten days apart.

Four of the rabbits were to be immunized against *B. coli* employing the rapid method of immunization recommended by Gay. Two injections only were given within a period of three days, for some of the rabbits lost weight and others died after the third injection with colon. After the first injection the leucocytes were to be counted. One of these rabbits immunized against *B. coli*, no. 73, was to receive typhoid vaccine after an interval of forty days, and twenty days later colon vaccine. Another, no. 72, received colon vaccine after an interval of forty days, and 20 days later typhoid vaccine.

Another, no. 70, received typhoid vaccine after an interval of ten days and colon vaccine after ten days. The last, no. 71, received colon vaccine after an interval of ten days and typhoid vaccine after an interval of ten days.

It was impossible to adhere strictly to the intervals mentioned above but the variations did not exceed two days.

The weights of the animals were carefully watched and in most cases there appeared an increase in weight. Rabbit 79, typhoid immune, having lost 200 grams after the injection with typhoid vaccine appeared emaciated the day he was counted but recovered before the next injection. Rabbit 72, colon immune, lost the same number of grams after an injection of typhoid vaccine. Rabbit 73, lost weight and died two days after the colon injection. These results indicate that the dosage of vaccine was large enough to cause a well marked reaction; impairing the health of the animals to a greater or less extent.

Four normal rabbits, two injected with typhoid vaccine and two with colon vaccine, all of which died during the process of immunization, showed a marked leucocytosis after the first injection of vaccine.

It was thought that our failure to secure the extreme hyperleucocytosis might have been due to the fact that we had employed an old laboratory strain of typhoid. Hence Rabbit 76, was given a strain isolated from a patient in bile culture medium a few weeks previously. This strain was grown on plain nutrient agar through a few transplants and was then kept on rabbit blood agar. The rabbit although previously immunized was given

three injections of this strain within a period of three weeks. The first injection was one loop of a living culture. The last a whole standard blood agar slant. Its agglutinins when tested showed a positive reaction in the dilution 1-10,000. Nine days after this large immunizing dose of *B. typhosus* it was injected with a whole

TABLE 10

HOURS AFTER INJECTION	RABBIT 84	RABBIT 80	RABBIT 69	RABBIT 68
	Normal	Normal	Normal	Normal
	Injected with B. Coli	Injected with B. Typhosus	Injected with B. Typhosus	Injected with B. Coli
Control	15,400		10,800	7,600
18	42,200	12,350	47,400	13,200
18½	42,400	9,000	33,500	15,400
19	37,000	10,550	28,120	11,800
19½	37,200		32,750	11,200
20		10,850	24,700	
20½	37,200	9,800		10,800
21	30,300		30,350	12,050
21½	30,300	10,600	29,400	11,500
22		8,400	26,200	8,200
22½	37,200			
23	36,000	8,000	31,950	10,800
23½				
24	37,400	6,600	28,150	10,400
24½	34,600		31,750	
25	35,400	5,400	33,450	12,600
25½			26,100	11,850
26	35,300	6,800	30,100	10,400
26½	38,400			10,200
27	38,200	6,200	24,800	11,150
27½	37,200		27,000	12,150
28	33,500		20,600	10,800
28½				9,600

slant of the living culture and the leucocytes were counted from the seventh to the twenty-seventh hour (see table 6). The leucocytic count is again much lower than that of Gay.

In connection with this work a rabbit highly immunized against the cholera vibrio was injected after a two months' interval of time with 1 cc. of a heavy suspension of live cholera organisms

grown twenty-four hours on nutrient agar. This animal also gave but a slight leucocytosis the result again challenging the theory of specificity of reaction.

Differential counts were made in a few instances at the time the quantitative changes of the leucocytosis were estimated. Normally the blood picture shows but few polymorphonuclears, about 30 per cent, and 60 per cent of lymphocytes. About eight hours after the injection the polymorphonuclears have increased to about 80 per cent and the lymphocytes have dropped to 19 per cent. With the increase and decrease of leucocytes

TABLE 11
Rabbit 119. Immunized against Cholera Vibrio

HOURS AFTER INJECTION	INJECTED WITH CHOLERA VIBRIO AFTER 60 DAYS	HOURS AFTER INJECTION	INJECTED WITH CHOLERA VIBRIO AFTER 60 DAYS
Control	11,400	23½	
18	18,400	24	14,400
18½	18,200	24½	
19	16,650	25	14,800
19½	15,000	25½	14,000
20	16,600	26	16,000
20½	16,000	26½	14,200
21	14,250	27	16,800
21½		27½	14,800
22	15,700	28	14,400
22½		28½	
23	15,400		

these percentages fluctuate, but the percentage of polymorphonuclears remains above normal until the count has begun to fall, about the twenty-seventh hour. This is in accord with observations made in typhoid fever cases injected intravenously with typhoid vaccine (4). The change in count is dependent upon the change in the total number of polymorphonuclears.

Table 12 is a summary of all the maximum counts in the preceding tables. Of seven rabbits that were counted first as normal after the injection of typhoid, later as typhoid immune, after the injection of typhoid, four showed almost exactly the same count in both instances. One showed a greater count after being

TABLE 12

		INJECTED WITH	MAXIMUM COUNT	INTERVAL
				<i>days</i>
Rabbit 96	Typhoid Immune.....	B. Typhosus	42,900	11
	Typhoid Immune.....	B. Coli	43,000	7
Rabbit 7	Typhoid Immune.....	B. Coli	32,000	7
	Typhoid Immune.....	B. Typhosus	16,600	5
Rabbit 86	Typhoid Immune.....	B. Typhosus	23,400	7
	Typhoid Immune.....	B. Coli	39,400	7
Rabbit 85	Normal.....	B. Typhosus	20,600	
	Typhoid Immune.....	B. Typhosus	22,400	39
Rabbit 19	Typhoid Immune.....	B. Coli	31,100	7
Rabbit 82	Typhoid Immune.....	B. Typhosus	22,700	41
	Typhoid Immune.....	B. Coli	29,200	20
Rabbit 93	Normal.....	B. Coli	52,400	
	B. Coli Immune.....	B. Typhosus	29,000	7
	B. Coli Immune.....	B. Coli	19,800	7
Rabbit 83	Normal.....	B. Coli	23,400	
	B. Coli Immune.....	B. Coli	67,400	42
	B. Coli Immune.....	B. Typhosus	27,200	40
Rabbit 79	Normal.....	B. Typhosus	14,450	
	Typhoid Immune.....	B. Typhosus	22,700	40
	Typhoid Immune.....	B. Coli	20,200	20
Rabbit 78	Normal.....	B. Typhosus	31,250	
	Typhoid Immune.....	B. Coli	26,500	41
	Typhoid Immune.....	B. Typhosus	30,750	21
Rabbit 77	Normal.....	B. Typhosus	46,800	
	Typhoid Immune.....	B. Typhosus	26,825	41
	Typhoid Immune.....	B. Coli	64,400	21
Rabbit 76	Normal.....	B. Typhosus	23,300	
	Typhoid Immune.....	B. Coli	18,600	42
	Typhoid Immune.....	B. Typhosus	23,250	19
	Typhoid Immune.....	B. Typhosus	26,100	9
Rabbit 75	Normal.....	B. Typhosus	36,500	
	Typhoid Immune.....	B. Coli	28,250	10
	Typhoid Immune.....	B. Typhosus	35,250	10
Rabbit 74	Normal.....	B. Typhosus	41,500	
	Typhoid Immune.....	B. Typhosus	25,900	10
	Typhoid Immune.....	B. Coli	44,400	12
Rabbit 73	Normal.....	B. Coli	19,000	
	B. Coli Immune.....	B. Typhosus	17,825	38
	B. Coli Immune.....	B. Coli	36,300	21
Rabbit 72	Normal.....	B. Coli	19,250	
	B. Coli Immune.....	B. Coli	15,600	40
	B. Coli Immune.....	B. Typhosus	21,975	19

TABLE 12—Continued

		INJECTED WITH	MAXIMUM COUNT	INTERVAL
				<i>days</i>
Rabbit 71	Normal.....	B. Coli	8,800	
	B. Coli Immune.....	B. Coli	24,600	10
	B. Coli Immune.....	B. Typhosus	22,500	12
Rabbit 70	Normal.....	B. Coli	20,600	
	B. Coli Immune.....	B. Typhosus	23,800	12
	B. Coli Immune.....	B. Coli	20,825	10
Rabbit 68	Normal.....	B. Coli	15,400	
Rabbit 84	Normal.....	B. Coli	42,400	
Rabbit 80	Normal.....	B. Typhosus	12,350	
Rabbit 69	Normal.....	B. Typhosus	47,400	
Rabbit 119	Cholera immune.....	Cholera vibrio	18,400	60

immunized and two showed greater counts when normal. Four additional typhoid immune rabbits that were not counted as normal were injected with *B. typhosus* and counted and showed that the average of their hyperleucocytic counts was slightly less than the average of the normal rabbits injected with *B. typhosus*. Ten rabbits immunized against *B. typhosus* were injected with both *B. typhosus* and *B. coli* at different intervals. One showed the same count after both injections, four showed greater counts after the typhoid injection, and five showed greater counts after the *B. coli* injection.

Six rabbits were injected with *B. coli* while normal and after having been immunized against *B. coli*. One showed the same count in both instances, two showed a greater count when normal, and three a greater count when immunized. Six rabbits immunized against *B. coli* were injected with both *B. coli* and *B. typhosus* at different intervals. One rabbit showed the same response after both injections, three showed a greater count after the typhoid injection, and two showed greater counts after the *B. coli* injection.

Typhoid bacilli were injected into typhoid immune rabbits six times at intervals of from seven to eleven days after the last previous injection and gave an average count of 38,000. Six were injected after an interval of nineteen to forty-two days after the last previous injection, and showed an average count of

35,000. Three rabbits immunized against *B. coli* were injected with *B. coli* at short intervals of time, seven to ten days, and gave an average count of 22,000; three were injected at long intervals of time, twenty-one to forty-three days after the previous injection and showed an average count of 40,000. With colon immune rabbits a greater leucocytosis was observed after the long interval of time but we are inclined to think that this result was accidental, since the typhoid immune rabbits showed practically the same degree of leucocytosis after the long interval as after the short one.

Two exceptionally high counts were obtained, both following the administration of colon vaccine. One was a typhoid immune rabbit, given an injection of colon vaccine twenty-one days after the injection of typhoid vaccine. The other was a colon immune rabbit given an injection of live colon vaccine forty-two days after the last immunizing dose.

Ten rabbits gave counts over 40,000. Five of these were normal, and five immunized.

The cultivation of the typhoid and colon bacillus upon ten per cent rabbit blood agar did not seem to have any more stimulating influence upon the leucocytic response than when both cultures were grown on plain agar; as many high counts were obtained after the injection of vaccine grown on plain agar as after that of vaccine grown on the new medium suggested by Gay.

Throughout all these experiments the results point to a leucocytic reaction of non-specificity. The above experiments seem to warrant the following conclusions:

1. Both normal and typhoid immune rabbits respond with a well marked leucocytosis to the intravenous injection of typhoid bacilli, but the response is not different in degree in the two instances.
2. Typhoid immune rabbits show the same grade of hyperleucocytosis when injected with *B. coli* as when injected with *B. typhosus*. The same is true of rabbits immunized against *B. coli*.
3. These results were obtained with killed and living vaccine, with an old laboratory strain of typhoid and a freshly isolated

one, and with typhoid bacilli grown upon plain nutrient agar and upon rabbit blood agar.

4. Hence no evidence has been observed in favor of the view that typhoid immune rabbits exhibit a specific hyperleucocytosis following the injection of vaccine.

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SCIENTIFIC PROCEEDINGS OF THE SOCIETY FOR SEROLOGY AND HEMATOLOGY, NEW YORK

February 9, 1916

1. STUDIES ON *TREPONEMA PALLIDUM* AND SYPHILIS

Hans Zinsser, J. G. Hopkins, and Malcolm McBurney: a. Treponemacidal Action of Immune Rabbit Serum on Culture Pallida. The experiments reported showed the normal rabbit serum was treponemacidal when as much as 1 cc. was used but seldom in smaller amounts, whereas the serum of animals immunized against cultures of treponemata showed the effect in amounts as small as 0.01 cc. This property of the immune serum was destroyed by heating to 56°C. for half an hour, and such inactivated serum could be reactivated by the addition of 0.05 cc. of normal serum.

b. Group Agglutinations of Culture Treponemata in Pallidum Antiserum. Serum obtained by immunizing rabbits against cultures of *treponema pallidum* agglutinated strains of this organism in amounts from 0.001 to 0.00025 cc. It showed an equally powerful action against the *treponema calligryum* and a weaker action against *T. refringens*, *T. microdentium* and against a small *treponema* isolated from the tonsil. It did not agglutinate *T. mucosum*. Absorption experiments showed that the pallidum and the calligryum absorbed both the major and the minor agglutinins, that the refringens and the microdentium absorbed only their respective minor agglutinins.

Agglutination tests on the serum of syphilitic rabbits showed that many animals which had been infected for a long period showed a slight increase of agglutinins for the culture pallidum as compared with normal animals, which was, however, very slight in extent when compared with animals artificially immunized.

c. Fluctuations of Virulence of Individual Strains and Comparative Virulence of Different Strains of Treponema Pallidum in Rabbits. Observations on five strains of *treponema pallidum* carried for from ten to twenty generations in rabbits showed no consistent differences in the virulence of the different strains or in the types of lesions which they produced. There was no progressive increase in virulence as shown by the percentage of takes or by the shortening of the incubation time in any strain in repeated animal passage. The percentage of takes, the length of the incubation period, and the character of the lesions produced, which have been attributed by other writers to differences inherent in the various strains of pallidum or to the increase in virulence on passage, were found to be due rather to the technique of in-

oculation, to the character of material used for inoculation, and to the size of the testicles in the inoculated animals.

d. Differences in Serum Susceptibility Between Virulent and Culture Pallida. Very marked differences were observed between the behaviour of virulent treponemata obtained directly from lesions in rabbits and that of treponemata which had been cultivated for a number of generations on artificial media and had completely lost their virulence for animals.

Agglutination experiments showed that the serum of rabbits immunized to cultures, which had a very powerful effect on the avirulent treponemata, agglutinated the virulent organisms very slightly or not at all. Treatment of the virulent organisms by the method of Porges did not render them susceptible to the immune serum. Treatment of virulent suspensions with immune serum before they were injected into animals showed no protective action on the part of the immune serum, although the serum showed in test tube powerful treponemacidal action against the avirulent organisms. This is more or less in keeping with the fact that infection with the treponemata excites little antibody formation. It is possible that a more definite effect on avirulent organisms would be obtained by more powerful immune serum.

e. Specific Complement Fixation and the Wassermann Reaction. (10 min.) The writers have utilized a method in their laboratory for the cultivation of treponema pallidum in large quantities for the production of antigen for complement-fixation. For the production of the antigen, slanted egg medium, as for Dorset's egg, is made up in high tubes, covered with ascitic broth, and sealed with paraffine oil. No tissue is needed for this and the egg can be thoroughly sterilized in the autoclave. The culture treponema grows actively on this so that plentiful growth can be obtained in periods as short as ten days. This enables one to work with young cultures. The material is centrifugalized, ground up with salt, and taken up in distilled water to isotonicity. The writers have paralleled the Wassermann reactions in 159 cases at the time of making this report, with entire identity in 138. There were 21 discrepancies, in a considerable number of which the treponema fixation indicated syphilis which the Wassermann missed. On the other hand, the writers do not regard their reactions, at present, as specific. Experiments indicate that similar antigens can be produced from egg cultures of colon and typhoid bacilli and that a certain amount of the fixation is due to the lipoidal content of the microorganisms. However, the apparently greater delicacy of this reaction may mean that there is a certain specific element superadded. It may also mean, however, that its reaction is more delicate only because a bacterial antigen produced in this way and very delicately titrated (and it is necessary in working with these antigens to approach more closely to the anticomplementary dose) represents simply another way of doing the Wassermann. This, the writers think, is quite probably the case in the many fixations they obtained with the treponema antigen in early syphilitic cases in which their other work does not indicate the existence of antibodies to any great degree.

Further work is being done in the direction of distinguishing the differences between the treponema antigen and similarly produced antigens from other bacteria, and fixations are being done with the protein and lipid fractions of the treponema antigen.

f. The Agglutination of Cultivated Treponema Pallidum by Normal and Syphilitic Human Sera. Tests were carried out with suspensions of culture treponemata mixed with varying dilutions of active human serum, in test tubes. The tests were incubated for one hour at 37°C., and allowed to stand in the ice-box over night. Readings were then made microscopically. In dilutions of 1:2—that is equal parts of undiluted serum and pallidum suspension—a large number of normal sera were found to agglutinate.

Of 40 sera from normal individuals examined, 1 agglutinated in 1:10 dilution; none above this. In 6 cases of primary syphilis, 2 agglutinated in 1:10, and none above this. In 19 cases of secondary syphilis, 10 agglutinated in 1:2; 8 in 1:10; 3 in 1:50; and 1 in 1:100. In 63 cases of tertiary syphilis, 32 agglutinated in 1:10; 4 in 1:50; and 2 in 1:100. The results so far indicated that there might be a slight antibody formation in secondary and tertiary cases, which could be detected by agglutination. In 39 sera, however, obtained from patients with non-syphilitic diseases, agglutination was obtained with 12 in 1:2, with 12 in 1:10, and with 3 in 1:50 dilutions.

In view of the strong group reactions which we have observed in treponemata, it might be that such reactions are due to infection with some treponema other than pallida; for example, to throat infections. The strong agglutination observed in a few of the cases tested seems to indicate that there is slight antibody formation in the late stages of syphilis. The observations, however, hold out little hope that the reactions can be of value in diagnosis.

2. A METHOD OF PRODUCING ANTIGEN FOR COMPLEMENT-FIXATION IN TUBERCULOSIS

H. R. Miller and Hans Zinsser: The writers have prepared an antigen for complement-fixation in tuberculosis for which they claim certain advantages over those prepared by previous workers, especially those of Besredka and Petroff, and the modifications of Besredka's antigen of Bronfenbrenner and Craig. One of the writers (Miller) has especially studied the Petroff antigen in connection with the one reported this evening. There is nothing very original about the method of preparation, which consists entirely of the endotoxin extraction method previously applied by Besredka to typhoid bacilli and other organisms; namely, the grinding up of a weighed amount of bacillary substances with salt, and its subsequent suspension in distilled water until isotonicity is obtained. The antigen so prepared is not anti-complementary in quantities as high as 1.0 cc. and has given positive fixations with quantities as low as 0.02 cc. It does not give positive Wassermann reactions with syphilitic sera; that is, it has failed to do

so in all but one case out of 26, and, of course, tuberculosis could not absolutely be excluded in that case. A total number of 190 cases thus far examined are reported, 93 of which were clinically negative cases based on careful clinical examination by attending physicians of three institutions, and in all of them the fixation test was negative. 24 of the 93 gave positive skin reactions, but were adults without clinical signs of tuberculosis. 89 cases gave positive reactions, and all of these were actively tuberculous, ranging in degree from the incipient to the very far advanced third stage. This communication is entirely in the form of a preliminary report, but the writers make it because of the regularity of the results in the hope that others will be stimulated immediately to try it.

3. GAS BACILLUS INFECTION WITH REMARKS ON GASTRIC ULCER

Randolph West and Mary E. Stewart: West and Stewart have studied a gas bacillus brought from the French front, particularly with regard to its toxin formation, since Weinberg and Sacquepée have recently described a true toxin from an organism belonging to this group. Cultural and morphological examination placed their organism distinctly in the "Welch bacillus" group. Filtrates of anaerobic broth cultures killed guinea pigs but it was found that this effect could not be attributed to a true toxin, since neutralization of the acid filtrate removed this toxicity. Suspicion of the toxic effect of the acid led to the injection intraperitoneally and intravenously of acid solutions equivalent to the acidity of the toxic filtrates of the cultures. The gas-bacillus infected guinea pigs, in the large majority of the experiments, showed gastric ulcers. Gastric ulcers were also noticed in the guinea pigs injected intraperitoneally and intravenously with acid. Gastric ulcer, without death of the animal, occurred regularly after intravenous injection of 4 to 4.5 cc. of 1 per cent acetic acid. The acid solutions were not as uniformly toxic as the acid filtrates, possibly because of the presence in the acid filtrate of butyric acid which is highly toxic.

THE EFFECT OF LECITHIN AND HORSE SERUM ON THE HEMOLYTIC ACTION OF CERTAIN PEPTONES

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In a preceding communication (1) it was shown that certain peptone fractions cause hemolysis and agglutination of different species of red blood cells. The peptones used in the tests were the individual fractions obtained by separation of Witte's peptone and those arising from the peptic digestion of casein. As stated there, corresponding peptone fractions from the two sources, possess similar properties, although in certain instances the reactions obtained differ in degree.

The hemolysis produced by the peptones is as follows:

Peptone B (Witte) hemolyzes ten species of red blood cells: Human beef, horse, goat, rabbit, guinea-pig, cat, dog, mouse and pig.

Peptone B (Casein) hemolyzes ten species of red blood cells: Human, beef, horse, goat, rabbit, guinea-pig, cat, mouse, goose and pig.

Peptone A (Witte) hemolyzes ten species of red blood cells: Human beef, horse, goat, sheep, rabbit, guinea-pig, dog, goose and pig.

Deuteroalbumose B (Witte) hemolyzes nine species of red blood cells: Human, beef, horse, goat, sheep, rabbit, guinea-pig, cat and goose.

Deuteroalbumose B (Casein) hemolyzes nine species of red blood cells: Human, beef, horse, goat, sheep, rabbit, guinea-pig, cat and dog.

Protalbumose (Witte) hemolyzes three species of red blood cells: Horse, goat and dog.

These results appeared strongly suggestive of the similarity of the peptone bodies to certain other hemolytic agents. A preliminary test made with the hemolytic peptones and lecithin,

disclosed the resemblance of the peptones to cobravenom. In view of the lasting controversy over the nature of the influence of lecithin upon cobravenom hemolysis, the results obtained with the peptones seemed worthy of further investigation.

The proteoses and peptones are secondary proteins. They are cocto-stabile and are devoid of all ferment action. There is no evidence available which indicates the presence of any lipolytic power in them. By a study of the conduct of the peptones towards red blood cells in the presence of lecithin, we acquire further information concerning the action of lecithin with similar hemolytic agents.

TABLE 1
Showing the effect of lecithin upon the hemolytic action of peptones

DILUTION OF THE PEPTONE BODIES	PROT- ALBUMOSE 0.2 cc.	HETERO- ALBUMOSE 0.2 cc.	DEUTERO- ALBUMOSE A 0.2 cc.	DEUTERO- ALBUMOSE B 0.2 cc.	PEPTONE A 0.2 cc.	PEPTONE B 0.2 cc.	ALCOHOL SOLUBLE PEPTONE 0.2 cc.	ALCOHOL INSOLU- BLE PEPTONE 0.2 cc.
In each tube 0.2 cc. lecithin 1-8000								
1-10	+++	0	0	+++	+++	+++	+++	+++
1-20	+++	0	0	+++	trace	+++	+++	+++
1-40	++	0	0	+++	0	+++	+++	+++
1-80	0	0	0	+++	0	+++	+++	trace
1-160	0	0	0	+++	0	+++	trace	0
1-320	0	0	0	+++	0	+++	0	0
1-640	0	0	0	+++	0	+++	0	0
1-1280	0	0	0	+++	0	++	0	0
1-2560	0	0	0	+++	0	0	0	0
1-5120	0	0	0	+++	0	0	0	0

The plus sign (+) indicate the degree of hemolysis. Guinea-pigs' red blood cells in 5 per cent suspension were used in the test.

In the first series of experiments an attempt was made to ascertain the general effect of the lecithin upon the different peptones (Table 1) and it was found that lecithin exerted a distinct influence upon the behavior of deuterioalbumose B and peptone B. As the accompanying tables (2 and 3) show, the hemolytic action of deuterioalbumose B can be advanced from a dilution of 1-80 (for guinea-pig's red blood cells) to one of 1-2500 and 1-5000, by $\frac{1}{10}$ of the hemolytic dose of lecithin.

The effect of lecithin upon peptone B is somewhat different from that of the deutoalbumose.

It was shown in the previous communication (referred to above) that in concentrated solutions, peptone B agglutinates and fixes sheep's red blood cells, so that they resist the solvent action of a number of agents, including that of distilled water. It was also shown that guinea-pig's red blood cells on the other hand were

TABLE 2

Deutoalbumose B {	1-20 dil.	1-40 dil.	1-80 dil.	1-160 dil.
control..... {	+++ hem.	++ hem.	+ hem.	0 hem.
Lecithin control.... {	1-400 dil.	1-800 dil.	1-1600 dil.	1-3200 dil.
Lecithin..... {	+++ hem.	+++ hem.	++ hem.	0 hem.
Lecithin..... {	1-6000 dil.	1-8000 dil.	1-10000 dil.	1-15000 dil.
Deutoalbumose.... {	1-5120 dil.	1-5120 dil.	1-5120 dil.	1-2560 dil.
Deutoalbumose.... {	+++ hem.	+++ hem.	+++ hem.	++ hem.

Remarks. The plus signs represent degree of hemolysis. Guinea-pig's red blood cells used in the test (5 per cent suspension). 0.2 cc. of each ingredient used in the hemolytic system; total quantity of fluid in each tube being 1.0 cc. Time of incubation = 30 minutes, temperature 37°C.

TABLE 3

Peptone B control... {	1-20 dil.	1-40 dil.	1-80 dil.	1-160 dil.
Peptone B control... {	+++ hem.	+++ hem.	+ hem.	0 hem.
Lecithin control.... {	1-400 dil.	1-800 dil.	1-1600 dil.	1-3200 dil.
Lecithin control.... {	+++ hem.	+++ hem.	++ hem.	0 hem.
Lecithin..... {	1-6000 dil.	1-8000 dil.	1-10000 dil.	1-15000 dil.
Peptone B..... {	1-2560 dil.	1-1280 dil.	1-320 dil.	1-320 dil.
Peptone B..... {	+++ hem.	++ hem.	+++ hem.	++ hem.

Remarks. The plus signs indicate the degree of hemolysis. Guinea-pig's red blood cells used in the test (5 per cent suspension). 0.2 cc. of each ingredient used in the hemolytic system; total quantity of fluid in each tube being 1.0 cc. Time of incubation = 30 minutes, temperature 37°C.

dissolved by peptone B, especially in the higher dilutions, and that after the hemolysis has taken place, a precipitation of the laked cell material occurs. In the present series of experiments it was found that when lecithin is added to peptone B and the mixture then applied to the red blood cells of sheep, the cells are no longer agglutinated, as before, but that a hemolysis occurs with a subsequent precipitation of the laked cell material.

It seems therefore that through the intervention of lecithin, the conduct of the peptone towards sheep red blood cells is rendered similar that which it bears to guinea-pig cells. This fact is of interest because it attests in a way to the correctness of the hypothesis that the difference in the susceptibility of sheep and guinea-pig red blood cells is dependent to a certain extent upon the difference in the lipid or lecithin content of the two species of blood cells.

TABLE 4

HORSE SERUM 0.2 cc.	DEUTERO- ALBUMOSE B (0.2 cc. OF 1-10 DIL.)	DEUTEROALBUMOSE B 0.2 cc.		
		Dilution	Alone	With horse serum 1-128 dil. 0.2 cc.
Dilution	Hemolysis		Hemolysis	Hemolysis
1-1	0	1-10	+++	+++
1-2	0	1-20	+++	+++
1-4	0	1-40	++	+++
1-8	0	1-80	+	+++
1-16	0	1-160		++
1-32	0	1-320		+
1-64	++	1-640		0
1-128	+++			
Guinea-pig red blood cells (5 per cent suspension) used. Showing the marked inhibition of the hemolysis of the albumose by means of horse serum. Hemolysis occurs only when the added serum is sufficiently dilute.		Guinea-pig red blood cells (5 per cent suspension) used. Showing the action of dilute horse serum on the hemolysis by albumose.		

The above deduction gains support from the following experiment. When the red blood cells are sensitized with non-hemolytic amounts of peptone, then washed free of the substance, and then minute amounts of lecithin are added to them, no hemolysis occurs. But when the cells are first treated with a similar amount of lecithin, the cells washed, and the peptone added, it is found that the hemolysis caused by the peptones is much greater than that which takes place with non-sensitized blood cells. It ap-

pears from this, that lecithin increases the vulnerability of the red blood cells, and renders them more readily soluble by the peptones. This heightened hemolytic activity is not due to a summation of the hemolytic action of the agents employed.

One fact pointed out by Michaelis and Rona (2) is of interest in this connection. They found that the peptones to which lecithin

TABLE 5

Horse serum

Whole: no hemolysis—1-2 dil. = no hemolysis.

Euglobulin:* 5 per cent solution in 0.9 per cent saline. Whole = no hemolysis. 1-2 dil. no hemolysis.

Pseudoglobulin:* 5 per cent solution in 0.9 per cent saline. Whole = ++ hemolysis. 1-2 = no hemolysis.

Albumin:* 5 per cent solution in 0.9 per cent saline. Whole = no hemolysis. 1-2 dil. = no hemolysis.

DEUTERO- ALBUMOSE B	A EUGLOBULIN		B PSEUDOGLOBULIN		C	
	1-2 dil. (i.e. = 2.5 %)	1-4 dil. (i.e. = 1.25 %)	1-2 dil. (i.e. = 2.5 %)	1-4 dil. (i.e. = 1.25 %)	Albumin	Deutero- albumose 1-10 dil.
	Hemolysis	Hemolysis	Hemolysis	Hemolysis	Dilution	Hemolysis
1-10	+++	+++	+++	+++	1-1	0
1-20	+++	+++	+++	+++	1-2	0
1-40	+++	+++	+++	++	1-4	0
1-80	+++	+++	+++	+	1-8	+
1-160	0	0	+	0	1-16	+++
1-320	0	0	0	0	1-32	+++
Showing slight activation of the albumose hemolysis.					Showing marked inhibition of the albumose hemolysis.	

* The serum fractions were obtained by precipitation with ammonium sulphate in the usual manner and the salt removed by dialysis.

thin is added are rendered soluble in chloroform. This is a striking point of similarity between the conduct of these bodies and that which characterizes cobralecithid. The author attributes the change in solubility of the peptones in the presence of lecithin to the physico-chemical character of these substances.

The question as to whether the peptones enter into chemical combination with lecithin and form a lecithid, has not been

determined in this study. It has been found that lecithin alters the hemolytic and agglutinative action of the peptones, in relation to different species of red blood cells.

It seems apparent that the increase in the hemolytic activity of the peptones resulting from the addition of minute amounts of lecithin is not due to the intervention of a lipolytic ferment and the subsequent splitting off of a fatty acid from the lecithin.

As in the case of cobra venom, the activation of the peptones can be accomplished not only by the addition of pure lecithin to them, but also by the addition of horse serum. The activation of the peptones with serum is not as marked as with the lecithin, and the effect does not become apparent until the serum is sufficiently diluted (table 4). Whole serum fails to activate the peptones and interferes with their hemolytic action. This is due to the inhibiting effect of certain serum proteins (Albumin-A, table 5). The serum after extraction with ether does not activate the peptones in any dilution. The lecithin that is firmly attached to the serum proteins fails to activate the peptones. Such serum can not be rendered active by digestion with pepsin and hydrochloric acid.

(1) EPSTEIN, ALBERT A.: *Jour. Exp. Med.*, 1912, xv, 485.

(2) MICHAELIS, L. AND RONA, PETER: *Bioch. Ztschr.*, 1907, iv, ii.

STUDY OF A STRAIN OF *B. WELCHII* ISOLATED IN FRANCE TOGETHER WITH SOME NOTES ON GASTRIC ULCERS

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1. INTRODUCTION

Weinberg and Sacquépée have recently announced the discovery of a strain of gas forming bacillus isolated from cases of gas gangrene, among the French soldiers, which gives rise to a true soluble toxin, and Weinberg has announced the production of an antitoxin. All previous investigators, both European and American, have agreed that the gas bacilli do not form a true soluble toxin although many hold that toxic substances may be elaborated by the bacillus on artificial media. On account of the great importance which an antitoxin might have in the treatment of gas gangrene, we have undertaken a careful study of one strain of a gas forming bacillus recently isolated from a case of gas gangrene in France.

The bacilli described by Weinberg and Sacquépée are very similar to the organism that we have studied, but differ from it in several particulars. Simonds quotes Schattenfroh and Grassberger to the effect that the butyric acid forming bacilli—to which group the gas bacilli belong—may be divided into three groups; the non-motile organisms, which do not form spores on fermentable sugar media; the motile organisms, which do form spores on the fermentable sugars; and the putrefactive organisms, which may be distinguished from the other two groups by their inability to cause the stormy fermentation of milk. *B. Welchii* is a member of the first group, and the organism here studied, being non-motile and unable to sporulate in the presence of fer-

mentable sugars, must also be considered as a strain of *B. Welchii*. The organisms of Weinberg and Sacqué́e are motile and sporulate on fermentable sugar media, therefore they cannot be considered as strictly identical with the above mentioned bacilli. They resemble the bacillus here described in being anaerobic and gram positive, in fragmenting dextrose agar with gas and in producing a fatal gas gangrene on intramuscular injection into guinea pigs.

2. MORPHOLOGY AND STAINING

The bacillus studied by us is a straight rod-like organism with rounded ends, somewhat longer and considerably thicker than *B. anthracis*. It occurs singly, in pairs or in short chains. There are, however, marked variations in morphology from short coccoid forms to single organisms which reach half across an oil immersion field. Many gram negative degeneration forms have been noted, including club, dumb bell and crescent shapes.

The bacillus stains readily with the usual aniline dyes, although more lightly stained areas have often been noted in some of the organisms. All of the younger cultures are gram positive, though in the older cultures gram positive and gram negative forms may be seen together. Capsules have been observed in the animal body, and in cultures. Spores were noted only on sugar free broth plus coagulated egg white and then only in the depths of the egg white.

3. BIOLOGICAL CHARACTERISTICS

The organism was found to be non-motile when examined in capillary tubes filled with a young clouded dextrose broth culture.

The bacillus is a strict anaerobe. It must be noted, however, that in agar stab cultures a certain amount of turbid fluid containing the bacilli appears on the surface of the medium, probably forced upward by the pressure arising from gas formation. Growth and gas formation have also been noted in aerobic tubes of dextrose, inulin and maltose broths. This is probably due to

the fact that these tubes were filled almost to the top with broth, and the bottom was therefore anaerobic. The growth on albumin covered broth was variable, and the only reliable results were obtained within Buchner tubes.

4. CULTURAL CHARACTERS

Surface colonies on dextrose agar resembled those described for *B. Welchii* by other observers. In glucose agar stab there occurred an abundant formation of gas which fragmented the medium. The agar was not liquefied. As other investigators have failed to note any diagnostic characteristics for *B. Welchii* on gelatin, we did not use this medium.

Litmus milk cultures all showed the typical stormy fermentation within forty-eight hours. Acid was formed, the milk was coagulated and filled with gas bubbles. There was a distinct odor of butyric acid from all milk and carbohydrate cultures.

There was no gas formation and no noticeable growth on sugar free broth. On 1 per cent carbohydrate broths dextrin, dextrose, lactose, maltose, saccharose and starch were fermented with an abundant gas formation, while glycerin and inulin yielded a decidedly smaller amount of gas. On mannite neither acid nor gas occurred. In every case in which gas was formed there was an abundant growth of the bacillus and the medium became acid to litmus.

In sugar free broth to which coagulated egg white was added there was a fair growth of the bacillus, the medium became slightly acid to litmus and a small but constant amount of gas was present. By the end of the fourth day the small cubes of egg white had begun to disintegrate.

The bacillus was injected intravenously into a rabbit and the animal killed a few moments later. After about five hours of incubation the rabbit was greatly puffed up; there were gas bubbles in the tissues, especially in the liver, and bacilli could be isolated from almost all parts of the body.

5. INJECTION AND PATHOLOGY

The injection of 2 cc. of a dextrose broth culture of the bacilli into the thigh muscles of a guinea pig gave rise to typical gas gangrene and the death of the animal in from six to eighteen hours.

At autopsy an abundant sero-sanguinous exudate was noted extending for some distance from the point of injection, containing almost no pus cells but laden with the bacilli in pure culture. There was an extensive muscle necrosis extending from the point of injection until it usually involved the entire leg and adjacent part of the abdomen. The muscle tissue was so necrosed that it had become semi-liquid and it was riddled with gas bubbles. There was a characteristic very offensive odor. Altogether eight guinea pigs were injected in the flank muscles with the gas bacilli. Three of these were killed early in the disease and only the local lesions already alluded to were noted. Of the other five animals, four of which were etherized and examined during the moribund period while still alive, all showed multiple ulceration of the gastric mucosa and hemorrhage into the stomach. In four instances, blood cultures were taken just before and just after death. They were twice negative, and twice the bacilli were recovered in pure culture.

Microscopically the infected muscle was found to be so greatly disintegrated that in places no structure could be recognized. The bacilli filled this necrotic area and extended from it along connective tissue planes about still intact muscle fibers, in many of which the nuclei stained poorly. Sections of the gastric ulcerations showed that the mucosa and submucosa were eroded but the muscularis and serosa were intact. The neighboring vessels were dilated, but there was no round celled infiltration nor were any bacilli seen in the gram stained sections. There was a swelling of the cells of the kidney tubules but very few epithelial cells were found in the tubule lumina.

6. TOXIC PRODUCTS

We have not been able to demonstrate that this organism forms any true soluble toxin nor has the bacterial protein been found to be toxic even in large doses. There are, however, certain filterable toxic products formed on artificial media and also, we believe, in the animal body.

In all subsequent experiments guinea pigs weighing from 200 to 300 grams were the only animals used and with but one or two exceptions they were examined either during the moribund period under ether or autopsied within a few minutes after death.

The effect of the bacillary protein was first investigated. Three forty-eight hour agar slants of *B. Welchii* washed and killed by heating to 72° were injected intraperitoneally into one animal, while another received a similar heat-killed *B. coli* culture as a control. The animals showed no symptoms aside from slight restlessness and when killed several days later they presented no lesions.

Ten cubic centimeters of the bacteria-free filtrate of a 72 hour dextrose broth culture of *B. Welchii* were injected into each of two guinea pigs while as controls one animal received a similar *B. coli* filtrate and another a like amount of uninoculated broth. The animal receiving the uninoculated broth remained well, while the three animals that were injected with the *B. Welchii* and *B. coli* filtrates died in six to eight hours. At autopsy there was marked congestion but no necrosis of the peritoneum, and multiple ulcerations of the gastric mucosa, the serous surface of the stomach being intact. The *B. Welchii* and *B. coli* filtrates, though derived from very different organisms, were found to have one property in common, their acidity. With both filtrates, about 4 cc. N/1 NaOH were required to render 100 cc. neutral to phenolphthalein, that is, they were 4 per cent acid in terms of N/1 acidity.

Accordingly two animals were injected intraperitoneally with 10 cc. of the neutralized *B. Welchii* filtrate and a third received a like dose of neutralized *B. coli* filtrate. The *B. Welchii* animals lived, but the *B. coli* guinea pig died three days later of a

general peritonitis following a perforation of the lower gut secondary to an intussusception that seemed to bear no direct relation to the injection. None of the animals had gastric ulcers when examined post mortem nor were any lesions found aside from the ones mentioned.

As the evidence up to this point indicated that acidity of the filtrates was chiefly responsible for their toxicity, the effect of the intraperitoneal injection of pure acetic acid—one of the acids formed by the bacillus—of the same strength as the acid filtrates was investigated. Two animals received 10 cc. each of a solution of acetic acid representing 4 per cent of normal solution (that is 0.24 per cent by weight) intraperitoneally. One guinea pig weighing 180 grams died in four hours and showed multiple ulcers of the stomach, the other weighing 260 grams survived and showed no gastric ulcerations when killed and examined. It must be remembered that the bacillus forms from carbohydrates not only the comparatively non-toxic acetic acid but also the relatively toxic butyric acid and traces of formic acid, and this may explain the more uniformly severe effects of the bacterial filtrates as compared with those of pure acetic acid. This experiment however, does not exclude the possibility of the direct action of the acid through the stomach wall, although the peritoneal surface of the stomach has always been found intact.

Accordingly pure acetic acid of varying strengths was injected into the external jugular vein. This had to be done very slowly, but even so eight of the animals died during the injection of the acid, two of them of hemorrhage, probably of the mesenteric veins, which filled the peritoneal cavity with blood. Twelve of the animals survived the injection. Of these, seven received less than 4 cc. of 1 per cent acetic acid and when killed showed no gross lesions. The remaining five animals each receiving from 4 to 4.5 cc. of 1 per cent acetic acid. All of these when killed four to twenty hours later showed multiple ulcerations of the gastric mucosa. The only associated lesions visible to the naked eye were two or three superficial yellowish areas of necrosis in the liver, about 5 mm. in diameter, in two of the

animals, and a congested condition of the lungs in one. No ulcers were seen in the intestines. One of the guinea pigs died of general peritonitis secondary to the perforation of several of the gastric ulcers, but the other four appeared to be normal and healthy when killed for autopsy.

The question at once arises as to whether the bacillus can elaborate enough acid to cause such injury in the animal body. In order to ascertain this, the necrotic infected muscle tissue was removed from a guinea pig just dead of *B. Welchii* infection and titrated with phenolphthalein as an indicator. It was found on several titrations at least 10 cc. of $N/1$ NaOH were needed to neutralize 100 grams of the muscle. Oppenheimer states in his *Handbuch der Biochemie* that normal guinea pig's muscle requires from three to five cc. $N/1$ alkali per 100 grams to render it neutral to phenolphthalein, depending on whether it is resting, fatigued, or in a state of rigor mortis. It will be remembered that the acid bacteria-free filtrates used in this work required 4 cc. $N/1$ alkali neutralize 100 cc. to phenolphthalein.

Two spontaneous gastric ulcers have been noted in a guinea pig in this laboratory during the last four months and although an exact count of the animals used is not available, the impression is that not more than one animal in twenty or thirty has a spontaneous ulcer. One series of twenty-four carefully examined animals revealed no ulcers of the stomach at autopsy.

In the gross the ulcers vary in size from 5 mm. to 3 or 4 cm., and in one guinea pig practically the whole anterior fundic wall of the stomach was perforated. In shape the ulcers are very irregular and but few resemble the typical "punched out" human gastric ulceration, nor are they limited to any one area of the stomach. Microscopically the lesion consists of an erosion of the epithelium usually extending no deeper than the muscularis mucosa. The surrounding vessels are dilated, but no thrombi nor bacteria have been noted in the sections. There is practically no round celled infiltration of the tissues of the stomach wall.

No duodenal nor other intestinal ulcerations have been found in any of the animals, although one or two have shown small subserous hemorrhages of the colon. Focal necrotic areas were

found in the livers of two animals injected intravenously with acetic acid.

The acid filtrates of this organism have been found to exert a marked hemolytic action on red blood cells, the products of hemolysis being brownish in color and not red. When neutralized no such action has been observed even when equal amounts of filtrate and 5 per cent sheep cell suspension were mixed and incubated at 37°. McCambell states that pure butyric acid will produce a similar hemolysis and the products of hemolysis on the addition of HCl to blood in the Sahli hemoglobinometer show the same brown color. Simonds has noted hemolysis in some neutralized *B. Welchii* filtrates.

We have made no direct experiments regarding negatively chemotactic substances, but the absence of pus cells in the *B. Welchii* exudates would favor the view that such products exist. McCambell has traced such effects to an acid action and has been able to show in a series of opsonic index experiments that phagocytosis of washed *B. Welchii* can be prevented either by the addition of unneutralized *B. Welchii* filtrate or by similar strengths of butyric acid.

7. SUMMARY AND CONCLUSIONS

The study of an anaerobic bacillus isolated from an infection in a French soldier has shown it to belong to the group of butyric acid bacilli classified as *Bacillus Welchii*. It has been found to produce no true exotoxin nor was the bacterial protein toxic. Nevertheless the bacillus has killed 5 pigs acutely, in spite of the fact that no general distribution occurred, and the bacilli were found in the blood stream at death either in small numbers, or not at all.

Since death could be produced in these animals by injection of the bacterial free filtrate, and the toxicity of this filtrate could be rendered neutral with sodium hydroxide we feel inclined to attribute much of the toxemia produced by the bacillus to its ability to form acid. Evidence has been advanced to show that large amounts of acid were formed in the lesions, probably by splitting of glycogen within muscle tissue.

We believe, therefore, that acid formation of *Bacillus Welchii* in the tissues, constitutes a powerful factor in the injury of the animal body.

Of the lesions distant from the focus of infection, the most striking one has been gastric ulceration. This has induced us to scrutinize closely the literature on experimental ulceration. As far as we can determine, only the acute, rapidly healing ulcers have been experimentally produced, and not the more chronic ones observed so frequently in human beings. These ulcers have been found to be produced through various agencies many of which are apparently unrelated to the question of acidity.¹ However, there are some experiments which more or less

¹ Rosenow's production of gastric ulcers by the injection of streptococci and his views on the specific localization of certain strains streptococci are too well known to be repeated here. Mann of Mayo's Clinic has noted the formation of gastric ulcerations in a large number of dogs and cats following double adrenalectomy. The ulcers developed in the absence of pancreatic juice or bile and could be prevented by continuous etherization or feeding with sodium bicarbonate. Mann quotes Durante as stating that section of the median or minor splanchnic nerve on either side is followed by ulcerations of the gastric mucosa, while Elliot has observed the formation of gastric ulcers in guinea pigs following the subcutaneous injection of tetra hydro- β -naphthylamine hydrochloride, a drug which causes rapid exhaustion of adrenalin from the glands by impulses passing over the splanchnic nerves. The ulcers could be prevented by feeding sodium bicarbonate. The injection of thyroid extract and adrenalectomy have produced gastric ulcers in the hands of Friedman and the same author has noted duodenal ulcers following the injection of adrenalin. This is especially interesting in the face of the fact that Fawcett, Rogers, Rahe and Beebe state that thyroid extract subcutaneously was the most potent stimulant of gastric secretion examined by them, while adrenal extract tended to lessen the gastric secretions. Interesting in connection with the adrenal work is the observation of Rosenau and Anderson that gastric ulcerations are not infrequent in guinea pigs killed with diphtheria toxin a substance causing marked adrenal lesions in these animals. Bolton has produced ulcerations of the gastric mucosa by injecting guinea pigs intraperitoneally with the sera of rabbits immunized with guinea pig gastric mucosa or liver. Finally Bardeen quotes Schjerning's statistics compiled from about two hundred autopsy reports, collected from the literature, of death following extensive burns. In those cases in which death occurred in less than forty-eight hours after the burn no duodenal ulcers were noted, but 19 per cent of those dying after forty-eight hours showed this lesion. Associated lesions were hyperemia of the brain, lungs and intestines, nephritis, pneumonia, pleurisy and meningitis. Bardeen observed five cases of extensive burns but all died within ten hours after injury and no duodenal ulcers were found.

parallel our own. Türekc has found gastric ulcers in dogs following the feeding of large quantities of broth inoculated with colon bacilli, which doubtless was acid in reaction. Bolton has produced ulcerations of the gastric mucosa in cats, monkeys and guinea pigs by feeding them with 0.7 to 1.6 per cent HCl. The gastric mucosa of the guinea pigs in this case, was invariably ulcerated after doses of 0.7 to 0.9 per cent HCl.

We have found that ulcerations of the stomach in guinea pigs occurred regularly after intravenous injections of 4 to 4.5 cc. of 1.0 per cent acetic acid.

In view of these facts, we feel justified in concluding that the excess of acid produced by the tissue necrosis is connected with the formation of the gastric ulcers, probably modifying the reaction of the blood stream. Whether the modified blood reaction causes the death or lessened vitality of many of the body cells and the subsequent digestion of these cells of the gastric mucosa which have been injured, rendering them noticeable as a gross lesion, or whether it is possible for the stomach through its acid forming mechanism to lessen the blood acidosis by markedly increasing the acidity of the gastric juice and thus bring about the ulceration of the mucosa, can only be determined by a further investigation.

Of course the ulcer formation, although interesting, is but an incidental lesion. The point we wish to emphasize is that this organism forms no true toxin, nor does it regularly invade the blood stream. It is the acid produced by the bacillus in the breaking down of tissue that is a factor of great importance in causing the death of the infected animal.

We believe that the important principle demonstrated by this work is the fact that severe injury can be produced within the animal body by products of the bacterial activities upon the body tissues; products which are in no sense bacterial poison, but represent substances derived from the cleavage of the tissue constituents. Libmann, some years ago, suggested this possibility in his work on the streptococci, but subsequent workers have, we believe, failed to take cognizance of this possibly important means of injury to the animal body.

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HUMAN SENSITIZATION

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PART I

I. INTRODUCTION

The data contained in this paper are collected from the histories of 621 cases of human protein sensitization observed during the past five years and summarized in the protocol herewith published.

We were stimulated to this work as the result of a personal experience of one of us in the fall of 1910 with the realities and

the possibilities of the protein reaction in sensitized individuals,—both its immediate dangers as well as its ultimate beneficial effect.

While not all of the histories cover all of the points discussed throughout the paper, extreme care has been used in eliciting them and only definite and positive facts are included. Any error, therefore, and errors undoubtedly do exist, is due rather to a lack of knowledge on the part of the patient and would appear on the negative rather than on the positive side in our statistics.

Human sensitization to foreign protein has only recently come to occupy an important place in the field of medicine although certain facts, such as the development of urticaria after the ingestion of particular foods, the production of an attack of bronchial asthma after exposure to certain animals and the seasonal occurrence of hay fever from pollen of grasses and plants have long been known. However, the true nature of these attacks as anaphylactic in nature has been thoroughly understood and generally accepted only for the past few years.

Hypersensitiveness to toxins, created experimentally in animals, had been remarked upon by Richet (1) who introduced the word "anaphylaxis" to designate this hypersensitive state as one strictly opposed to prophylaxis and distinctly disadvantageous to the host. In 1903 Theobald Smith, while working with diphtheria antitoxin, first observed the phenomenon that guinea-pigs previously injected with serum died suddenly in convulsions on the readministration of this serum, if a sufficient time had elapsed between the two injections.

Extended studies to elucidate this phenomenon were then undertaken by Otto (2) in Ehrlich's laboratory and by Rosenau and Anderson (3) in this country. Both of their articles, representing independent work, appeared in 1906. In 1906 Wolff Eisner (4) suggested that the clinical manifestation, known as hay fever, was anaphylactic in nature. Meltzer (5), in 1910, further suggested that bronchial asthma was of the same nature, a suggestion based largely on the complete clinical analogy between the reaction occurring in the guinea-pig experimentally and that in human beings during their attacks.

II. DISCUSSION OF THE ANAPHYLACTIC NATURE OF CERTAIN CLINICAL MANIFESTATIONS IN THE HUMAN

There are two conceptions by which we might explain those clinical manifestations known as urticaria, angio-neurotic edema, bronchial asthma, hay fever and certain attacks of acute gastro-enteritis. Either they are due to a toxin, or they are due to a protein reaction in sensitized individuals; in other words, anaphylaxis.

As far as hay fever is concerned, Dunbar maintains that the protein of the pollen is a toxin belonging to the albumen group. On this basis, however, it seems impossible to account for the peculiar hypersensitiveness of those individuals who are suffering from hay fever. For such cases give positive ophthalmic and cutaneous reactions with amounts of pollen protein inconceivably small, whereas normal cases show no reaction to doses of 100 and even 1000 times that amount.

One of Ehrlich's postulates for a toxin is that its physiological action be practically identical for all individuals of the same species. This we now know is not strictly true, for about 50 per cent of normal adult human individuals do not react on the introduction of diphtheria toxin in small amounts. This variability in the reactivity has been proved both experimentally and clinically to be due to an immunity as a result of the presence of natural antitoxin in those cases that do not show a reaction. This principle is applied today in the so-called Shick test of immunity to diphtheria.

If, then, we accept the toxin theory for pollen protein and explain the variations of reactions on this basis, it must be demonstrated that the absence of hay fever in any individual is due to the presence of a natural antibody to the pollen toxin. This, however, is not the case. The striking difference between the two reactions is this: In persons that do not react to diphtheria toxin, a natural antitoxin has been found; whereas in those that do not react to pollen protein, antibodies to this substance are *not* present. And, conversely, those that react positively with diphtheria toxin do *not* have antibodies to this toxin, while cases

of hay fever that do react to pollen protein are believed to have the specific antibodies present in their blood, as claimed by Clowes (5) who employed the precipitan and complement-fixation reactions for their demonstration.

If, then, we cannot accept the toxin theory as the cause of these reactions in man, the positive proof of their anaphylactic nature must lie in the fact that specific antibodies are continually present in the cells and at times in the blood of those affected with any of the clinical manifestations we are discussing.

Koessler (6) states that he has been able passively to sensitize guinea-pigs to ragweed pollen, using the blood of an individual afflicted with late hay fever. Assuming, then, that any individual, for reasons still unknown, has become naturally sensitive to some certain protein, the production of symptoms is readily understood. It has been definitely proved by the researches of Dale, Manwaring, Pearce and Eisenbrey, Weil (7), Coca and others that the anaphylactic reaction is cellular and not essentially humoral in character. For this reason, in human sensitization, the clinical type of the reaction depends entirely upon the cells that are sensitized and we have in hay fever an essential sensitization of the mucous membrane of the eyes, and the respiratory tract together with a general cutaneous sensitization; whereas in urticaria the sensitization is essentially in the cells of the epithelium. In angio-neurotic edema the sensitization is of the connective tissue cells in the subcutaneous tissue, whereas in those cases of acute gastro-enteritis from the ingestion of certain foods it is the cells of the mucous membrane of the intestinal tract that manifest the reaction.

Furthermore, it is manifestly impossible for us to consider these clinical manifestations, occurring in certain groups of patients, as due to toxins, for on this basis it would be necessary to conclude that all normal human beings are immune to their normal food proteins; in other words, that the proteins of our food are toxic unless we carry bodies immune to them. For this reason again, therefore, we must decide against the toxin theory as an explanation for the reactions that are observed in certain individuals, and we can, on the other hand, satisfactorily explain

them as anaphylactic; in other words, certain individuals become and remain naturally sensitized to various proteins that in themselves are not toxic, but which on introduction into the sensitized individual show the specific reactions.

III. PATHOGENESIS

Since human sensitization to such peculiar and varied forms of protein so frequently occurs, it is not only interesting but important to study its pathogenesis.

Inheritance has been frequently, yet somewhat vaguely mentioned as a causative factor in hay fever, which is undoubtedly the commonest form of human sensitization. We have thought it worthy of study, therefore, to determine definitely what part inheritance plays, not only in hay fever but also in those other clinical manifestations of sensitization—bronchial asthma, urticaria, angio-neurotic edema and acute gastro-enteritis following the ingestion of certain foods such as fish and strawberries—and we have made it a particular point in preparing our family histories to inquire as to all these reactions. We have excluded, however, eczema, a history of which, it would be well to state, is commonly elicited, and migraine and epilepsy until further observation might warrant their inclusion. We have, therefore, studied sensitization as a whole and have included as positive in our family history all those cases in which the above mentioned reactions have taken place.

1. The frequency of human sensitization

Without its having any direct bearing upon the pathogenesis of sensitization, but because the statistics for its determination were at hand from a group of normal persons, that we had taken at random, we deemed it of interest to determine the frequency of human sensitization in general.

Of 68 individuals questioned, five gave a history of sensitization. In this group, inquiry was limited to father and mother and the group therefore includes three times 68 or 204 cases, 14 of whom, or 7 per cent gave a clinical history of sensitization.

In general, therefore, we may say that the frequency of human sensitization with clinical manifestations is probably not over 10 per cent.

2. *Inheritance as a factor in sensitization*

Excluding from our 68 cases the 5 that were themselves sensitive, we have 63 cases, apparently normal, that give a history of sensitization in the father or mother six times, or 9.5 per cent. Seventy-six other normal persons gave a positive antecedent (direct or collateral), family history eleven times, or 14.5 per cent.

Among our 621 cases of sensitization, the antecedent, direct, or collateral history was negative in 260, positive on one side in 205, positive on both sides in 39 and discarded as incomplete in 117 (table 1). In other words, of 504 cases with satisfactory history there was positive antecedent, direct or collateral history in 48.4 per cent. This is in striking contrast to the 14.5 per cent of positive antecedent histories in 76 normal persons and it would

TABLE 1*

Showing a list of the cases grouped according to their inheritance

A. Bilateral family inheritance history—39 cases

22, 72, 73, 106, 116, 134, 187, 292, 307, 310, 314, 317, 319, 333, 341, 342, 367, 372, 380, 385, 390, 399, 400, 401, 436, 437, 441, 442, 461, 468, 486, 540, 545, 551, 570, 571, 572, 602, 623

B. Unilateral family inheritance history—205 cases

3, 5, 31, 32, 35, 37, 39, 41, 42, 43, 45, 47, 50, 57, 60, 62, 63, 65, 67, 70, 76, 77, 78A, 78B, 80, 82, 90, 91, 93, 103, 104, 107, 108, 109, 114, 121, 122, 125, 127, 129, 131, 144, 149, 150, 151, 153, 155, 156, 158, 160, 166, 167, 168, 169, 172, 175, 176, 177, 178, 179, 182, 184, 188, 190, 199, 203, 205, 207, 210, 211, 213, 215, 216, 217, 220, 221, 224, 226, 228, 229, 230, 233, 234, 236, 243, 248, 256, 257, 258, 266, 268, 277, 283, 286, 288, 291, 299, 306, 308, 312, 315, 316, 318, 323, 324, 325, 327, 330, 331, 332, 337, 338, 343, 344, 345, 346, 350B, 352, 354, 355, 356, 359, 363, 373, 384, 391, 392, 393, 394, 398, 403, 407, 408, 412, 414, 416, 417, 419, 425, 428, 430, 435, 438, 440, 445, 448, 455, 457, 458, 459, 460, 464, 470, 472, 474, 475, 476, 477, 478, 479, 481, 495, 497, 500, 505, 506, 508, 511, 512, 514, 516, 517, 519, 524, 527, 531, 533, 534, 537, 543, 546, 548, 552, 553, 555, 564, 566, 574, 577, 579, 580, 581, 582, 587, 590, 595, 597, 601, 603, 605, 612, 613, 614, 618, 622.

* Numbers in all the tables refer to history numbers in the protocol.

TABLE 1—*Continued*

C. Negative family inheritance history—260 cases

8, 9, 10, 12, 13, 14, 16, 18, 19, 20, 21, 23, 24, 25, 26, 28, 33, 34, 36, 38, 44, 46, 52, 53, 54, 55, 59, 61, 64, 66, 68, 69, 71, 74, 75, 79, 81, 83, 84, 85, 86, 87, 88, 89, 92, 96, 98, 99, 101, 110, 111, 112, 115, 118, 119, 120, 124, 128, 132, 135, 138, 143, 145, 146, 147, 154, 157, 159, 161, 162, 163, 164, 165, 170, 171, 173, 180, 181, 183, 185, 186, 193, 194, 196, 198, 201, 202, 204, 208, 209, 212, 218, 222, 225, 227, 232, 237, 238, 239, 240, 241, 242, 244, 245, 246, 247, 249, 250, 251, 252, 253, 254, 255, 259, 260, 261, 263, 267, 269, 270, 272, 274, 275, 276, 278, 279, 280, 281, 282, 284, 287, 293, 295, 296, 298, 300, 301, 302, 304, 305, 313, 321, 322, 326, 329, 334, 335, 336, 339, 340, 349, 350A, 353, 357, 358, 360, 361, 362, 365, 366, 368, 371, 375, 376, 379, 382, 383, 386, 387, 388, 395, 402, 404, 405, 406, 409, 410, 411, 413, 415, 420, 421, 422, 423, 424, 426, 427, 429, 432, 433, 434, 444, 446, 447, 449, 450, 451, 453, 454, 456, 462, 463, 465, 467, 471, 473, 480, 482, 483, 485, 487, 488, 489, 491, 493, 494, 496, 498, 499, 503, 509, 510, 513, 515, 518, 521, 523, 525, 526, 528, 532, 538, 539, 544, 556, 558, 559, 560, 565, 567, 568, 573, 575, 586, 589, 592, 594, 598, 600, 604, 607, 608, 609, 610, 615, 616, 617, 619, 620, 621.

seem to warrant the conclusion that inheritance is a definite factor in human sensitization. This being the case, how further may its influence be shown?

3. *Heredity and age of onset of clinical symptoms*

In order to study the influence of heredity upon the age of onset, we have divided our cases into three groups. First, those in which the antecedent direct or collateral history was negative; secondly, those in which such history was positive on one side and thirdly, those in which it was positive on both sides.

There are 500 cases in which the age of onset is given together with the complete antecedent family history. These cases are summarized in table 2 and more graphically represented in Chart I.

Unfortunately, the double inheritance group is small—only 44 cases, yet the difference is so striking that it is deemed best to include it. With three exceptions, cases 116, 292 and 468, at least one parent is involved. The accompanying chart is plotted according to the percentages at the different ages. For the cases of double inheritance the period of maximum liability is in the first five years when 36.3 per cent develop symptoms. For single inheritance cases, it is the age from 10 to 15 while the

TABLE
Showing a list of cases grouped according

	1-5 YEARS	6-10 YEARS	11-15 YEARS	16-20 YEARS
A. Negative inheritance, 253 cases.....	66, 74, 145, 159 186, 302, 305, 365 446, 447, 489, 510 619	10, 24, 25, 52 55, 85, 87, 98 132, 196, 281, 295 300, 304, 321, 326 335, 336, 340, 361 387, 405, 423, 426 429, 435, 487, 503 509, 567, 568, 586	13, 53, 75, 101 157, 163, 171, 181 194, 198, 209, 238 240, 245, 249, 260 280, 360, 366, 404 432, 456, 471, 473 528, 560, 573, 607 621	18, 59, 79, 84 99, 119, 180, 183 193, 202, 225, 237 244, 246, 252, 253 255, 357, 358, 371 379, 395, 402, 413 480, 496, 539, 565 592
Number of cases.....	13	32	29	29
Per cent of total.....	5.1	12.6	11.5	11.5
B. Unilateral inheritance, 203 cases.....	32, 35, 57, 62 65, 70, 176, 177 199, 203, 217, 221 233, 248, 277, 291 308, 312, 350B 393, 445, 495, 531 548, 597, 601, 613 614	5, 37, 47, 50 63, 93, 114, 144 153, 160, 175, 205 230, 256, 258, 299 313, 324, 325, 327 331, 337, 343, 352 407, 408, 440, 455 457, 505, 508, 512 582, 612, 618, 622	41, 60, 78A, 78B 82, 121, 122, 127 155, 167, 179, 188 211, 220, 228, 266 316, 347, 354, 356 392, 394, 419, 425 428, 435, 458, 470 472, 474, 475, 477 497, 500, 534, 537 543, 566, 574, 579 581, 587, 595, 605	31, 76, 80, 104 107, 172, 182, 184 229, 236, 257, 265 288, 306, 359, 363 373, 384, 398, 412 417, 517, 527, 564 590, 216
Number of cases.....	29	36	44	25
Per cent of total.....	14.3	17.7	21.7	12.4
C. Bilateral inheritance, 44 cases.....	187, 292, 317, 319 342, 436, 442, 486 551, 570, 623, 385 158x, 419x 487x, 192x	307, 310, 333, 399 400, 401, 437, 461 540, 572, 602, 360x 390	314, 367, 545	22, 72, 372, 380
Number of cases.....	16	13	3	4
Per cent of total.....	36.3	29.9	6.8	9.0

* The sign x following history numbers refers to descendants of patients: see "other relatives" column

2*
to their age of onset and their inheritance

21-25 YEARS	26-30 YEARS	31-35 YEARS	36-40 YEARS	41-45 YEARS	OVER 45 YEARS
19, 20, 44, 61 68, 69, 71, 81 96, 112, 115, 154 161, 185, 218, 232 239, 241, 242, 250 270, 274, 276, 334 349, 353, 375, 376 383, 388, 406, 420 422, 434, 483, 493 498, 538, 575	8, 21, 26, 46 64, 83, 88, 92 111, 128, 143, 146 162, 165, 173, 201 222, 247, 267, 278 284, 322, 329, 386 410, 421, 433, 450 525, 526, 558, 559 594, 604, 609	12, 14, 16, 28 33, 110, 118, 120 164, 204, 212, 227 259, 261, 269, 272 275, 279, 296, 409 454, 462, 467, 482 488, 494, 499, 513 515, 521, 523, 544 598, 600, 610, 620	9, 86, 89, 135 147, 251, 282, 287 293, 350A, 368, 411 415, 444, 449, 453 463, 465, 491, 532 589, 616	34, 254, 298 424	50, 36, 38 124, 170, 208 263, 301, 362 427, 451, 556 608, 617
39 15.4	35 13.9	36 14.0	22 8.8	4 1.6	14 5.5
39, 42, 43, 125 131, 150, 169, 178 190, 213, 215, 315 318, 332, 344, 355 416, 438, 448, 479 481, 511, 516, 524 533, 546, 555, 580	45, 77, 90, 103 109, 156, 158, 207 234, 283, 286, 345 403, 464, 478, 514 519, 552, 577	67, 108, 129, 149 168, 226, 330, 414 430, 506	91, 151, 243, 346 476, 603	224, 338	166, 210, 323 391
28 13.8	19 9.3	10 5.0	6 3.0	2 1.0	4 1.8
73, 106	116	441, 468, 571	134, 341		
2 4.5	1 2.2	3 6.8	2 4.5		

in protocol.

height of the curve in those cases with negative history is not reached until a still later age—20 to 25.

Inheritance, therefore, does exert a distinct effect upon the age of onset of symptoms of sensitization; the more complete the inheritance, the earlier the manifestation.

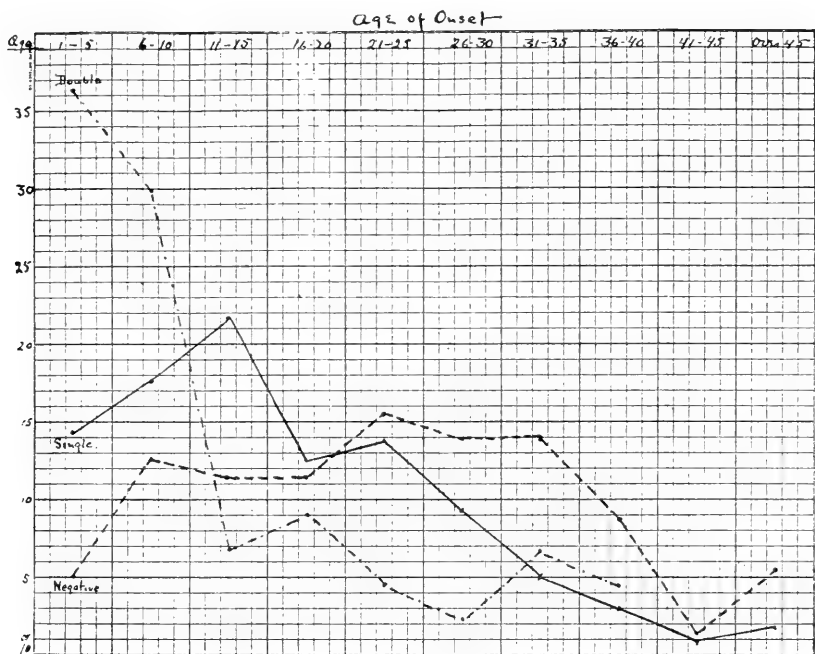


CHART I

4. The nature of the inheritance

Since it is shown that inheritance is a factor, the question naturally arises, of what does the inheritance consist? Is there the transmission of a definite and specific sensitization from parent to child; or does the offspring inherit merely the capacity, or the tendency to form specific antibodies to any form of protein of and by himself.

The following arguments are presented in favor of the latter hypothesis:

a. *The offspring of a sensitive parent are not born sensitive.* If a child actually inherits a specific sensitization, for example, a sensitization to grass pollen, it would be natural to expect that this sensitization would be apparent, clinically at least, by the second or third summer. But we know that this is usually not the case, for in those instances where one parent only is affected the clinical manifestations do not as a rule develop until the age of 10 to 15 years.

b. *A parent may transmit a tendency to sensitization without himself being sensitized.* It has been found that in cases of bilateral inheritance a larger proportion of the children become sensitized at an average earlier age than in the cases of unilateral inheritance notwithstanding the fact that in more than one-third of the former group the hereditary influence on one side of the family was not seen in the parent, but in a grandparent or a collateral, such as an uncle. It is apparent here that the parent not clinically affected has transmitted some characteristic to his offspring the nature of which cannot be specified.

It is however conceivable that such parents have a latent sensitization. By this we mean that while these individuals have none of the clinical symptoms to be interpreted as anaphylactic yet they would show a reaction if the proper protein were parentally introduced.

Longcope has shown, and we can substantiate his findings, that a fair number of apparently normal individuals will give positive reactions to proteins intradermally introduced, but such individuals do not have and never have had any clinical manifestations of such reactions, and this can be readily explained on the ground that these proteins as such, never reach the sensitized cells.

c. *Experimental evidence.* Another fact that speaks strongly against the direct inheritance of definite sensitization is furnished in the study of the transfer of hypersusceptibility from parent to offspring in guinea pigs. Otto (2) has shown that the young of anaphylactic mothers are passively sensitized for as long as 44 days. Rosenau and Anderson (3) also have shown this transfer of susceptibility from sensitized mothers, but this is

merely the passive transfer of a specific sensitization—of only short duration during the first few days of life and not transferable to a third generation; and further it has never been shown experimentally that the sensitized father transmits a

TABLE 3

Giving a list of the cases grouped according to the relation of the sensitization in the antecedent to the descendant

A. Relation of sensitization to parent

PATIENTS						PATIENTS. BROTHERS AND SISTERS					
Same		Inclusive		Different		Same		Inclusive		Different	
Father	Mother	Father	Mother	Father	Mother	Father	Mother	Father	Mother	Father	Mother
41	31	3	50	39	5	144	73	372	531	41	106
125	73	63	76	60	32	150	178	612		60	158
203	82	131	108	72	35	256	210			60	210
226	93	144	122	90	57	257	210			72	380
317	109	150	151	106	121	327	354			72	380
327	127	153	158	168	155	517				106	394
338	230	190	176	175	169	517				347	398
408	354	213	177	187	178	614				363	441
436	394	236	210	188	199					372	512
505	579	256	216	248	220					380	571
517	602	257	307	299	266					380	
		308	317	314	310					401	
		333	319	345	323					441	
		380	333	363	324					441	
		385	350B	372	330					540	
		401	380	390	342					546	
		407	385	399	343					548	
		437	412	400	390					571	
		442	442	430	448						
		486	458	440	486						
		524	461	441	512						
		548	472	540	533						
		574	479	546	540						
		582	495	551	543						
		602	508	572	551						
		612	511	581	553						
		614	531	587	580						
			537	601	595						
			572		613						
			618								
11	11	27	30	28	29	8	5	2	1	18	10

TABLE 3—Continued

B. Relation of sensitization to grandparent

PATIENTS			PATIENTS. BROTHERS AND SISTERS		
Same	Inclusive	Different	Same	Inclusive	Different
22, 41, 199 307, 319, 372 477	57, 67, 70 109, 216, 233 319, 319, 359 384, 458, 475 486, 545, 548 570	184, 317, 401 512, 612, 613	477	359, 359, 372	41, 67, 359 372, 401, 468 512, 548, 612
7	16	6	1	3	9

C. Relation of sensitization to uncle and aunt

PATIENTS			PATIENTS. BROTHERS AND SISTERS		
Same	Inclusive	Different	Same	Inclusive	Different
57, 62, 78B 144, 167, 182 187, 199, 211 221, 307, 399 400, 445, 477 477, 419, 497 514, 534, 566 577	17, 37, 47 78A, 129, 144 155, 166, 169 179, 190, 199 199, 199, 187 258, 292, 306 308, 312, 319 344, 352, 354 354, 355, 359 393, 401, 401 436, 436, 437 437, 438, 458 470, 481, 519 531, 570, 602 618, 486	35, 65, 109 149, 156, 172 151, 187, 187 188, 205, 211 283, 307, 310 314, 317, 317 317, 317, 319 319, 331, 332 333, 342, 346 372, 399, 399 400, 400, 416 417, 419, 436 437, 442, 457 458, 461, 486 570	144, 179, 393 445, 477, 477 534	354, 359, 359 372, 531	47, 47, 78A 172, 182, 221 283, 283, 359 372, 401, 401 416, 417, 468
22	44	43	7	5	15

sensitization even to the second generation. The situation here, then, is very different from that observed in the human being, and while it is not denied that placental sensitization may occur, in human beings certainly it is not usual.

d. Comparison of the sensitization of antecedent and descendant. The final and perhaps crucial argument in settling the point against the inheritance of a definite and specific sensitization is shown in tables 3 and 4. That placental sensitization is not an

TABLE 4

SUMMARY OF TABLE 3

Showing the relation of the sensitization in the descendant to that in the antecedents

ANTECEDENT	SAME				INCLUSIVE				DIFFERENT				TOTAL
	Pt.	Bor. S.	Both	Per cent	Pt.	Bor. S.	Both	Per cent	Pt.	Bor. S.	Both	Per cent	
Father.....	11	8	19	22.5	27	2	29	34.5	28	18	36	43.0	84
Mother.....	11	5	16	18.6	30	1	31	36.0	29	10	39	45.4	86
G'parent.....	7	1	8	19.0	16	3	19	45.2	6	9	15	35.8	42
Collateral*...	22	7	29	21.3	44	5	49	36.0	43	15	58	42.7	136
Collateral†...	16		16	20.0	31		31	39.2	32		32	40.8	79

* Uncles and aunts—gt. uncles and great aunts.

† Same as "a" but in families where parent is not affected.

important factor is evidenced by the fact that the inherited character is as frequently paternal as maternal and that in cases where the inheritance has been maternal, the clinical form in the child is much more apt to be different from the mother (45.4 per cent) than it is to be identical (18.6 per cent) (see table 4) and is no more apt to be identical with the mother than with the father.

The apparently normal parent whose antecedents or whose sisters and brothers are sensitized does transmit something to the offspring as discussed under Section 6. The interesting fact here is that in children whose uncles or aunts are sensitive the clinical type is just as apt to be identical with that of the uncle or aunt as it is to be like the parent.

Furthermore, we have among our cases the histories of six pairs of twins, summarized as follows:

CASE NO.	ANTECEDENT SENSITIZATION	TWINS	
		a	b
144	Pat. and mat.	Late h. f.	Same
336	Neg.	Urtic.-fish	norm.
393	Pat. uncle	Early h. f.	norm.
500	Mat. g'mother	Late h. f.	Same
622	Mat. aunt	Asth.-horse	norm.
629	Pat. g't aunt	Early and late h. f.	Same

Three of the six pairs have identical sensitization, but in none is the mother herself affected and in only two is the inheritance maternal alone and in these two pairs the sensitization is identical once and different once. Such facts certainly do not substantiate any claim for an inheritance of a specific sensitization.

To sum up then we must say that the results of a clinical study compel us to conclude that sensitized individuals transmit to their offspring not their own specific sensitization but an unusual capacity for developing bioplastic reactivities to any foreign proteins. This is a most important conception as a prelude to the following section.

5. Laws governing inherited sensitization

We have studied our cases of double, single and negative inheritance to determine whether or not they might conform to the Mendelian laws either as a dominant or as a recessive characteristic.

In the cases with double inheritance both parents being involved we find 26 families with a total of 70 children, 39 of whom show a sensitization clinically at the average age of 23 years. From our age of onset curve we know that 82.6 per cent of such cases have developed their sensitization at this age; therefore 39 is 82.6 per cent and 47.2 is 100 per cent, or the total number that will develop sensitization and 47.2 is 67.5 per cent of the total number of 70.

In the group with single inheritance we have 52 families with 150 children, 77 of whom are clinically sensitized at the average age of 27.5. From the age of onset curve we know that 84.5 per cent of these single inheritance cases will have developed symptoms at this age, therefore 91.2 children or 60 per cent of the 150 will become sensitive.

In contrast with this we have 148 cases with negative family history. Among 631 children there are 205 sensitive at the average age of 35 years. In the same way we figure that 244 will become sensitive. This is only 38.6 per cent of the total number.

TABLE 5*

Giving the list of cases with bilateral parental inheritance

CASE NO.	NUMBER OF CHILDREN	NUMBER SENSITIZED	TOTAL AGES	CASE NO.	NUMBER OF CHILDREN	NUMBER SENSITIZED	TOTAL AGES	CASE NO.	NUMBER OF CHILDREN	NUMBER SENSITIZED	TOTAL AGES	CASE NO.	NUMBER OF CHILDREN	NUMBER SENSITIZED	TOTAL AGES
72	3	3	88	390	4	1	173	572	3	1	55	404x	1	1	4
73	3	2	108	401	3	2	65	602	1	1	10	479x	1	1	6
106	5	2	181	441	3	3	99	385	2	1	18	487x	3	1	6
307	6	1	72	442	2	1	11	156x	2	0	16	548x	1	0	8
317	1	1	1	540	2	2	26	212x	2	0	11	571x	3	3	75
333	4	1	141	551	1	1	12	360x	2	1	33				
380	3	3	76	571	5	4	241	368x	3	2	42				

Number of families..... 26

Total children..... 70

Sensitized children..... 39

Average age of total children..... 23

* The sign x following history numbers refers to descendants of patients.
See "other relatives" column in protocol.

TABLE 6

Giving the list of cases with unilateral parental inheritance

CASE NO.	NUMBER OF CHILDREN	NUMBER SENSITIZED	TOTAL AGES	CASE NO.	NUMBER OF CHILDREN	NUMBER SENSITIZED	TOTAL AGES	CASE NO.	NUMBER OF CHILDREN	NUMBER SENSITIZED	TOTAL AGES	CASE NO.	NUMBER OF CHILDREN	NUMBER SENSITIZED	TOTAL AGES
5	5	3	62	226	1	1	38	403	2	1	93	508	5	1	53
31	2	1	89	299	3	1	68	407	4	2	78	511	2	1	62
35	2	1	71	324	3	1	28	412	1	1	26	512	3	2	116
42	1	1	36	325	2	1	30	414	2	1	85	517	3	3	94
50	1	1	50	327	2	2	19	428	2	1	96	524	1	1	37
57	2	2	66	337	1	1	24	438	4	1	142	431	4	2	33
60	5	3	170	338	3	1	158	440	3	1	18	546	2	2	57
131	2	1	53	354	4	2	83	448	3	1	77	548	5	2	229
144	3	2	148	363	4	2	77	458	1	1	36	553	2	1	52
169	3	1	62	373	3	2	92	472	3	1	72	495	6	1	158
178	8	2	288	394	4	2	105	474	3	1	90	579	4	2	100
203	2	1	35	398	3	2	77	505	1	1	26	581	2	2	68
601	2	1	7	612	2	2	52	614	5	2	75	618	4	2	143

Number of families..... 52

Total children..... 150

Sensitized children..... 77

Average age of total children..... 27.5

TABLE 7
SUMMARY OF TABLES 5 AND 6

Showing the percentage of sensitized children estimated to develop in the three inheritance classes

INHERITANCE	NUMBER OF FAMILIES	TOTAL CHILDREN	CHILDREN SENSITIZED		AVERAGE OF AGE OF TOTAL	% SENSITIZ. AT AVERAGE AGE	ESTIMATED SENSITIZED CHILDREN	
			No.	% Total			No.	% Total
Both parents.....	26	70	39	55.7	23.0	82.6*	47.2	67.5
One parent.....	52	150	77	51.3	27.5	84.5	91.2	60.0
Negative.....	148	631	205	32.4	35.0	84.0	244.0	38.6

* Based on Chart I.

In dealing with human beings the conditions are not precise enough for absolute accuracy. It is not possible to be sure that an individual is pure-sensitized or mixed. If the sensitization trait is pure in one parent *all* the offspring should be sensitized whether the trait in the other parent is pure or mixed. We have

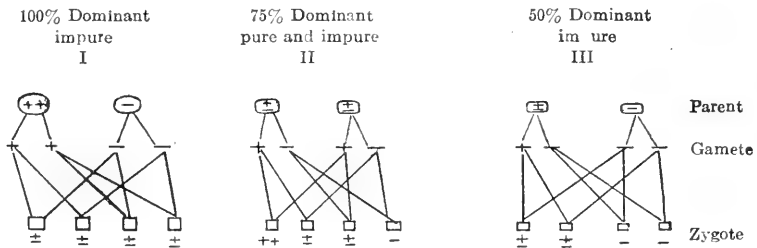


CHART II

$\pm\pm$ = Dominant pure. \pm = Dominant impure. $-$ = Recessive

no family sufficiently large and with all children sensitized, to be sure that this is the case. Assuming then that all parents though clinically sensitized, are impure with respect to the sensitization trait; that is, carry both sensitized and non-sensitized gametes, we should expect according to the Mendelian laws to have 75 per cent of the offspring sensitive when both parents are affected, and 50 per cent of the offspring sensitive when one parent is affected. These theoretical facts are shown graphically in Chart II. The figures obtained—67.5 per cent instead of the theo-

retical 75 per cent for bilateral inheritance, and 60 per cent instead of 50 per cent for unilateral inheritance are sufficiently close, strongly to suggest that sensitization is inherited as a dominant characteristic. With regard to those cases of sensitization in which there is an absence of sensitization in the family history, nothing can be said. We can only surmise that here the failure to find the antecedent sensitization was due to the limitations of our methods of ascertaining the family histories in this respect.

IV. MULTIPLE SENSITIZATION

We were, from the very first, impressed with the fact that a large number of our cases had clinical evidence of more than one sensitization. As far as hay fever itself is concerned, we find that 30 per cent of 530 cases have both the early and late variety, and of the total number of 551 cases of sensitization 42.3 per cent are multiple. These facts can be more concretely shown as follows:

A.	Early hay fever.....	96 cases	= 18	per cent
	Late hay fever.....	276 cases	= 52	per cent
	Early and late hay fever.....	158 cases	= 30	per cent
B.	With 1 sensitization.....	318 cases	= 57.7	per cent
	With 2 sensitizations.....	172 cases	31.2	} = 42.3 per cent
	With 3 sensitizations.....	42 cases	7.6	
	Over 3 sensitizations.....	19 cases	3.5	
			530	

In this list there were 37 sensitive to horse, 35 to strawberry, 28 to shellfish and 27 to fish.

V. ARTIFICIAL SENSITIZATION

The mere fact that over 42 per cent of a large number of sensitization cases are clinically multiple in type suggests very strongly the fact that such individuals as a group, possess a very peculiar capacity to become sensitive in a natural way to certain proteins to which their environment and habits of life frequently expose them, and reasoning from analogy then we

might expect that any form of foreign protein parenterally introduced would readily render them acutely sensitive. On account of the frequency with which horse serum is injected in the passive immunization to diphtheria, it has been possible to select a number of sensitization cases that have been previously injected with horse serum in varying amounts and over varying periods of time. We have applied the intradermal test of horse serum to these cases in order to decide whether or not they have become and remained anaphylactic to the foreign protein, which has been introduced in much larger amounts than ever could be the case with those forms of protein that are absorbed from mucous membranes either respiratory or gastrointestinal and to which persons have become naturally sensitive.

We know from the studies of Von Pirquet and Schick that normal individuals form antibodies to parenterally introduced serum and these are demonstrable after the tenth day and persist for from two to six months, as shown by an immediate cutaneous reaction to the specific serum. When, however, the interval between the first injection and the test was over seven months no immediate cutaneous reaction was obtained. There was, however, an altered reactivity as shown by an "accelerated" reaction in from five to seven days. As a basis of comparison for our sensitization cases we have applied the intradermal test with horse serum to 16 persons whose family and personal history showed no sensitization. They had received horse serum as antitoxin in the amounts and at the previous times indicated in table 8A.

None of the cases gave very strikingly positive cutaneous reactions though 7 of the 16 showed distinct response within one hour. In these 7 cases the last previous injection varied from four years to about seven weeks and all but one of these cases had received more than one injection. Four of the 7 had a marked reaction at the site of injection with the first dose, only one however had any constitutional reaction and that began on the eighth day.

Of the 9 cases that gave a negative cutaneous reaction only one had received two injections, these having been made at the short interval of three days, and only two had received fairly large amounts. All but two of the cases however had been injected

TABLE 8

Showing that naturally sensitized individuals (B) are not more prone to artificial sensitization than normal individuals (A)

A. Normal individuals				
PREVIOUS INJECTION	AMOUNT	REACTION AT SITE OF INJECTION	CONSTITUTIONAL REACTION	INTRADERM* TEST
1. 10 yrs. ago.....	Unknown	None	None	
Dec. 19, '14.....	1000 units	None	None	Pos. mild
2. Dec. 16, '14.....	5000 units	None	None	
Dec. 19, '14.....	5000 units	None		Pos. mild
3. Oct. 1, '14.....	1000 units	M'k'd 4 days	None	
Dec. 29, '14.....	1000 units	M'k'd 3 days	None	Pos. mild
4. 4 yrs. ago.....	2 injections	M'k'd 3 days	None	Pos. mild
8 yrs. ago.....	1000 units	M'k'd 1 week	Gen. urtic begin. Sth day	
5. 1 year ago.....	1000 units	M'k'd 1		Pos. mild
6. Dec. 29, '14.....	1500 units	Slight		
Jan. 5, '15.....	5000 units	Mod. 2 days		
Jan. 12, '15.....	5000 units	M'k'd 2 days	None	Pos. mild
7. 1 year ago.....	1000 units	Slight 2 days	None	Pos. mild
8. Dec. 19, '14.....	1000 units	M'k'd 2 days	None	Neg.
9. Dec. 19, '14.....	1000 units	Neg.	None	Neg.
10. Dec. 19, '14.....	1000 units	M'k'd 2 days	None	Neg.
11. 4 yrs. ago.....	1000 units	Slight	None	Neg.
12. Dec. 19, '14.....	1000 units	None	None	Neg.
13. Jan. 12, '15.....	10,000 units	Slight 1 day	None	Neg.
14. Dec. 19, '14.....	1000 units	Slight	None	Neg.
15. Feb. 10, '15.....	5000 units	Mod. 2 days	None	Neg.
Feb. 13, '15.....	5000 units	Mod. 2 days	None	Neg.
16. Dec. 19, '14.....	1000 units	Slight	None	Neg.

B. Naturally sensitized individuals					
CASE NUMBER	CLINICAL DIAGNOSIS	ANTIGEN	HORSE SERUM RECEIVED	CONSTITUTIONAL REACTION	HORSE SERUM INTRADERMAL TEST
313	Early and late hay fever	R. and G. Grass pollen	13 yrs. ago 5000 units antitoxin	None	Pos. marked
408	Early and late hay fever	Grass pollen Ragweed	2 yrs. ago 10,000 units antitoxin	None.	Pos. slight
577	Late hay fever	Ragweed	11 yrs. ago antitoxin	Urtic. 10 d. after injec.	Pos. mod.
612	Late hay fever	Ragweed	5 yrs. and 1 yr. 10,000 units each	Urtic. 10 d. after 2nd injec.	Pos. mod.
41	Late hay fever	Goldenrod and ragweed	3 yrs. ago tetanus antitox	None	Neg.
167	Late hay fever	Ragweed	4 yrs. ago 5000 units antitox	None	Neg.
183	Early hay fever	Grass pollen	4 yrs. ago 1000 antitox	None	Neg.
290	Bron.-asth.	?	6 yrs. ago antitoxin	None.	Neg.
314	Early hay fever	Grass pollen	10 yrs. ago 35,000 unit antitox	None	Neg.

* The intradermal tests were made March 14, 1915.

TABLE 8—Continued

CASE NUMBER	CLINICAL DIAGNOSIS	ANTIGEN	HORSE SERUM RECEIVED	CONSTITUTIONAL REACTION	HORSE SERUM INTRADERMAL TEST
325	Early hay fever	Grass pollen	antitox. 1 dose	None	Neg.
335	Early hay fever	Grass pollen	10 yrs. ago 2 doses antitox	None.	Neg.
407	Late hay fever	Ragweed	6 yr. ago 5000 unit antitox	None	Neg.
457	Late hay fever	R. and G.	13 yrs. ago 1 dose antitoxin	None	Neg.
474	Late and early hay fever	Grass and rag weed	14 yrs. ago 1500 units antitox	None	Neg.
487	Late hay fever	Ragweed	7, 6, 5, and 2 yrs. ago 1000 units each time	Immed. urtic. after last dose	Neg.
540	Bron.-asth.	?	4 yrs. ago 1 dose antitox	None	Neg.
597	Early and late hay fever	Grass and R.	6 yrs. ago 1000 units antitox	None	Neg.
A. W.	Bron.-asth.	?	4 mos. ago 25 cc. horse serum	No urtic	Neg.
M. W.	Early hay fever and asthma	Grass pollen	2 mos. ago 1000 units	No urtic.	Neg.

within eleven weeks. Two of the cases had marked reaction at the site of injection but none developed any constitutional manifestation.

In table 8 B are summarized the results of the tests on those cases that have shown the capacity to become naturally sensitized to proteins but who have no asthma or "hay fever" from exposure to horse. They have received subcutaneous injections of horse serum, in the amounts and at the times specified.

Of these 19 cases tested, all but three have been found to react positively to some form of protein that gives clinical symptoms. In several of the cases the amount of horse serum injected was rather large and several have had repeated doses. Only two showed a constitutional reaction on the tenth day. Four gave positive reactions to the cutaneous test with horse serum, only one being marked.

Although the cases are too few to permit absolute conclusions, they certainly indicate that artificial sensitization even in individuals with an abnormal capacity to sensitize, is not easy of accomplishment. Evidently the type of clinical sensitization

that they acquire and maintain is dependent upon some other factor than the mere parenteral injection of native heterologous protein.

PART II. HAY FEVER

Although hay fever was vaguely recognized from the middle of the sixteenth century, to Bostock must be given the credit for its description in 1819, as a disease with definite symptomatology and seasonal occurrence.

Elliotson in 1833 first suggested the pollen of the grasses as an etiological factor for that type of the disease occurring in England, and in 1872 Wyman stated that the ragweed pollen was the cause of another type of the same disease occurring in America. Finally Blackley (8), a victim of the disease, proved conclusively that the pollen of the grammaceae was responsible for the disease by the fact that he could induce a typical attack at any time of year by injecting himself with extracts of the pollen. Dunbar (9) confirmed the pollen theory and amplified the work by a thorough and painstaking study of the toxic action of the pollens of many of the grasses, plants and trees.

That hay fever is an anaphylactic reaction has previously been discussed. The disease manifests itself clinically in two forms, which we will designate as Early Hay Fever with a course from the middle of May to the first or middle of July and Late Hay Fever, beginning the middle of August and ending with the advent of frost.

I. SPECIFIC CAUSES OF HAY FEVER

Accepting, then, the anaphylactic nature of the reaction in hay fever and the pollen protein as that to which individuals with the disease are sensitized, we must determine which of the pollens are important causative factors.

Dunbar has published a long list of those that he has found active. The following is a list of those that we have found to give a positive cutaneous reaction with certain of the cases of early hay fever.

<i>Common Name</i>	<i>Botanical Name</i>
Timothy	Phleum pratense
Red-top	Agrostis alba
June grass	Poa pratensis
Sweet vernal	Anthoxanthum odoratum
Low spear	Poa annua
Orchard grass	Dactylis glomerata
Rye	Secale cereale
Wheat	Triticum sativum
Quickgrass	Agropyrum repens
Locust	Robinia pseudo-acacia
Chestnut	Castanea dentata
Maple	Acer rubrum
Daisy	Chrysanthemum leucanthemum
Rose	Rosa
Honeysuckle	Lonisera caprifolium
Privet	Ligustrum vulgare

The following is a list of those pollens to which the late cases give positive reaction:

<i>Common Name</i>	<i>Botanical Name</i>
Ragweed	Ambrosia trifida
Ragweed	Ambrosia artemisiifolia
Goldenrod	Solidago canadensis
Goldenrod	Solidago nemoralis
Chrysanthemum	Chrysanthemum
Dahlia	Dahlia
Zenia	Zenia
Clematis	Clematis Virginiana
Marsh grass	Spartina stricta
Aster	Aster

This list is indeed far from complete, for Dunbar has given 25 of the grasses besides 8 of the Cyperaceae and a large number of other plants. But the above list does include practically all of those which by reason of their prevalence and ubiquity are important inciting agents of the disease in this country.

An important question arises as to the specificity of these various pollens. We have tested a group of 20 cases of early hay fever with the pollen proteins of 6 of the most common grasses in order to determine whether or not there is any difference in the reaction. The following is a table of these cases with the results of the test:

TABLE 9

Showing group sensitization to pollen of grasses

CASE NO.	TIMOTHY	RED TOP	RYE	ORCHARD	WHEAT	JUNE
112	+++ *	++	+	+	++	++
111	+	+			+	++
110	+	+	+	+		+
107	++	++	+++	+++		++
103	+	+	+	+		+
109	++	++	++	++		
99	+	+	+	+		+
100	+	+	++	++	++	++
163	+	+	++	+	+	+
162	+	+	+	+	+	+
90	++	++	++	++	++	++
151	++	++			++	++
146	++	++	+	+	+	+
143	+-	+			+-	+
159	+	+	+	+	+	+
65	+++	++	+	+	+++	+++
108	+	+	-		+-	
115	++	++			+	+
157	+-	+-	+		+	

* Plus (+) signs refer to intensity of reaction.

An examination of this table shows that with very few exceptions a sensitiveness to one was associated with a sensitiveness to all. There are, to be sure, some minor variations in the degree of the reaction, but this is easily accounted for by slight differences in the amounts injected for the test. On the whole, we can say that an individual reacting to one reacts to all, which bespeaks a biological identity of the proteins derived from the pollens of the graminaceae. A further proof of this identity that can be mentioned here is the fact that immunizing injections with the pollen of one grass gives clinical relief of symptoms in those cases that have reacted to all and have from time to time been purposely exposed to that form of grass to which they were not specifically immunized.

As between the pollens of the grasses and various other plants and trees flowering at the same time, the case is distinctly different. Whether an individual is sensitive to any of the pollens

other than those of the grass is entirely a question of individual idiosyncrasy, and such sensitiveness requires separate immunization.

We have also tested a group of cases subject to late hay fever, using the pollen proteins of the two prevalent varieties of ragweed and the two more prevalent varieties of goldenrod. These two varieties of ragweed are botanically different and not subject to cross-fertilization in the open. The same is true for the varieties of goldenrod. We have collected in a table (table 10) a group of cases that have been tested with these four pollens.

TABLE 10

Showing group sensitization to the pollen of ragweed and golden rod

CASE NUMBER	AMBROSIA (Ragweed)		SOLIDAGO (Golden rod)	
	Trifida	Artemisiaefolia	Canadensis	Nemorialis
278	++*	++	+	+
54	++	++	+-	+
284		+++	++	
267	++	++	+	+
272	+	+	+	+
14	++	++	+	+
251	++	++	+	+-
268	++	++	+	+
46	+	-	+-	+-
285	++	++	-	-
280	++	++	++	++
260	++	++	+	+
286	++	++	+	+
224	++	++	+-	+-
275	++	++	+-	+-
26	++	++		
270	++	++	-	+-
287	+	++	+	+-
255	++	++	+	-
276	++	++	-	-
279	++	++	+-	-
273	++	++	+-	+-
196	+-	+		
211	++	++	+-	+
289	+-	++	-	
283	++	++	-	-

* Plus (+) signs refer to intensity of reaction.

This table shows that of the 26 cases tested those that reacted positively to one of the ragweed pollens, reacted in practically identical degree to the pollen of the other ragweed. The same holds true as between the two varieties of goldenrod; but it does not hold true between ragweed and goldenrod as 6 of the cases were absolutely negative to goldenrod while showing a positive ragweed reaction. From this we may infer that the pollens of ragweed are biologically identical, while botanically different, and the same holds true for the pollens of goldenrod. This is quite in keeping with the findings of Wells and Osborne (10) who were able to demonstrate the biological identity of the gliadins from wheat and rye and the hordein of barley.

II. THE SPECIFIC TREATMENT OF HAY FEVER BY ACTIVE IMMUNIZATION

With the exception of a few early and abortive attempts at active immunization, Dunbar was the first to attempt this procedure in human beings. He used solutions of the pollen protein that in the light of our present knowledge were many hundred times too strong. He obtained such violent reactions, with the production of attacks of hay fever, asthma, urticaria and edema that he soon desisted from these attempts and actively immunized horses, obtaining from them his "antitoxic" serum known as "pollantin," with which he has been able to obtain certain results in cases of hay fever.

It is known that the parenteral introduction of a protein into the animal body gives rise to the production of specific antibodies. If the injections are few and small, only a few antibodies are formed. If the injections are repeated over a sufficient length of time and are large enough, there is an excessive formation of the antibodies which then exist not only attached to cells but also circulating free in the blood and in the other body-fluids.

Under the usual experimental conditions of subcutaneous, intravenous, or intraperitoneal injection, Weil (7) has shown that the absence of anaphylactic symptoms following subsequent in-

jections may be due to the free, circulating antibodies which unite with the antigen and protect the cellular antibodies. And he has also shown that the immunity is only relative, for the introduction of a sufficient amount of antigen will always produce symptoms.

Under the conditions that exist in hay fever, with the antigen being absorbed directly by the sensitized membrane, all the existing theories of protection are unsatisfactory. It is not known at the present time exactly how an immunity is produced in such a condition as hay fever. It is not known whether the protection is due to an excess of free, circulating antibodies, or whether a state of desensitization exists. Experiments to determine the exact nature of the protection in hay fever are now being undertaken.

Koessler has reported, without however publishing the protocols of his work, that he was able passively to sensitize guinea pigs, using the serum from two patients suffering with asthmatic attacks in the third week of their hay fever. All of his animals on reinjection at the end of twenty-four hours, showed typical symptoms of anaphylactic shock. He also states that "guinea pigs can be readily sensitized to pollen protein and succumb on reinjection with typical anaphylactic shock." This work has not yet been confirmed but if this is so we have excellent proof that the pollen protein reactions in the human are identical with the protein reactions in experimental anaphylaxis.

Noon (17) was the first to publish observations on the treatment of hay fever by active immunization. The work begun by him has been continued by Freeman (16). Of necessity, it was confined to the early type of the disease. They used the pollen of timothy and extracted the protein by alternate freezing and thawing in distilled water and, after filtering, boiled the extract for ten minutes in sealed, glass tubes. In order to standardize their product they chose as a unit that "quantity of pollen toxin which can be extracted from the thousandth part of a milligram of phleum pollen." They measured the resistance of the patient by determining the strength of pollen extract necessary to excite a conjunctival reaction; and they found that as a result of the

subcutaneous injection the immunity as tested by the conjunctival reaction had increased one hundred fold.

In 1913 Clowes (15) reported on 8 cases of hay fever of the autumnal type; he used as his unit the soluble constituents of one-twenty millionth of a gram of pollen ($1/20,000,000$) and he was able to demonstrate in one case a thousand-fold increase in the resistance of the patient as measured by the ophthalmic reaction.

From this time on many reports appeared in the literature from workers in this field, among whom were Koessler, who reported on 41 cases, Ulrich (11), Goodale (12), Oppenheimer (13), as well as one of the authors (14) of this paper.

Both the ophthalmic and cutaneous reactions have been used to determine the quality as well as the degree of the sensitization. A drop of a very weak solution of pollen protein instilled into the eye of an individual sensitive to this protein produces a hyperemia of the conjunctiva and cornea, sometimes going on to edema and associated with a more or less intense degree of itching. The cutaneous reaction may be carried out as the von Pirquet test; that is, by rubbing a solution of the pollen, or even the pollen itself into a small scarified area. A more accurate method, however, is to inject the solution in amounts of $1/50$ or $1/100$ cc. intradermally. In all positive cases there develops within five to thirty minutes an urticarial wheal, usually with irregular pseudopod-like injections into the skin, the wheal being surrounded by a distinct zone of hyperemia 1 to 3 cm. in diameter. Usually there is a distinct sensation of itching.

In the application of active immunization in cases of hay fever the question of dosage is of the utmost importance. We know from the experience of Dunbar that violent and even dangerous manifestations may result from an overdose.

Is there any method other than empiricism to guide us in determining the dose that it is safe to use? Noon and Freeman have used the ophthalmic reaction as the indicator of their increased resistance in the patient, but they state that they were unable to use it as a guide to determine dosage. Clowes states that the ophthalmic test "can be used quantitatively and affords

a fair index of the measure of immunity." He does not state in just what way it can be applied to the question of dosage or to the intervals between doses. Koessler has not found it a safe index for determining the strength of the ensuing dose. The precipitin and complement-fixation reactions also will not suffice, for Clowes has shown that these reactions were exhibited only by a part of the cases and that when present before treatment the reactions disappeared under treatment, only to reappear after its cessation. The cutaneous test likewise is of no value. It is not sufficiently accurate, nor sufficiently sensitive.

Our patients under treatment when fully immunized and free of symptoms during the usual period of their attack still show a marked positive intradermal reaction. If there is any change at all the reaction in the immunized individual is somewhat more abrupt and the wheal lasts a shorter time and usually the sensation of itching, which was present before immunization, is markedly diminished or altogether absent. But these variations are comparatively slight and of no therapeutic value. In this respect we cannot confirm the findings of Oppenheimer who obtained a disappearance of the wheal after treatment.

The preparation of pollen extracts

The extraction of the pollen protein is comparatively simple. It is soluble in distilled water, the extraction being aided by freezing and thawing as suggested by Dunbar, or it can be extracted in concentrated salt solutions or normal salt solution. Noon followed the method of extraction adopted by Dunbar and standardized his extract by weight of the pollen. His unit was that quantity of pollen protein that can be extracted from the thousandth part of a milligram of pollen. Clowes is not specific, except to state that he precipitates with acetone and extracts with water. He does not mention how the solution for precipitation is prepared. Koessler extracts the protein in an 8.5 per cent NaCl solution. Both Clowes and Koessler have followed the method of Noon, standardizing by weight but adopting an individual and arbitrary unit.

Authors' method. With our earliest work in the extraction of pollen protein in 1911 we used a solution of 0.8 per cent NaCl in $\frac{N}{100}$ NaOH. The pollen or the polleniferous portion of the flower was ground in a mortar to a fine pulp with sand and the extracting solution. It was then shaken 24 hours, filtered, precipitated with 3 volumes acetone and redissolved in 0.8 per cent NaCl solution, to which 0.25 per cent trikresol was added. It was then filtered through a sterile Berkefeld filter and standardized according to its nitrogen content with the use of the ordinary Kjeldahl method. For the last two years we have employed simple extraction with 0.8 per cent NaCl omitting the acetone precipitation as unnecessary. For purposes of testing, both with the cutaneous and the ophthalmic methods, our solutions are made up in 3 strengths—0.01 mgm. N per cubic centimeter, 0.1 mgm. N per cubic centimeter and 0.5 mgm. N per cubic centimeter. For subcutaneous injections a solution containing 0.2 mgm. N per cubic centimeter is most convenient. In every way we have found this method of extraction and standardization eminently satisfactory, yielding solutions that are apparently of identical toxicity and immunizing capacity.

Active immunization in hay fever

By means of the intradermal test it is first determined to which pollen an individual is sensitive. Where positive reactions are obtained the different dilutions of such pollens (beginning with the weakest) are employed in the ophthalmic test to determine the degree of the sensitization. Where a positive ophthalmic reaction is obtained with a solution containing 0.01 mg. N per cubic centimeter, the first dose subcutaneously is 0.005 mg. N. If the ophthalmic reaction can not be obtained with a solution weaker than 0.5 mgm. N per cubic centimeter, the first dose is 0.02 mg. N.

Immunization in hay fever can be carried out either prophylactically or phylactically:

Prophylactic treatment. This is begun about six weeks before the expected onset of symptoms, and the injections are continued at approximately weekly intervals to within ten days or

two weeks of the end of the usual course. As a rule 10 to 12 injections are given in any individual case.

Phylactic treatment. Cases that are seen shortly before the usual onset or during an attack are given the original small first dose on four successive days. After an interval of three days a slightly larger dose is given, and injections are continued in gradually increasing strength and with a gradually lengthening interval up to a week, when injections are continued at weekly intervals to within ten days or two weeks of the expected termination of the disease.

It has been our experience that the most sensitive individuals—those that give positive ophthalmic reactions with solutions containing 0.01 mgm. N per cubic centimeter—respond most readily and require smaller doses throughout in order to have protection, while the cases that are the least sensitive require a much larger dose. This fact would seem to speak against a state of desensitization or antianaphylaxis as the determining factor in the protection afforded by the treatment.

III. REACTIONS: LOCAL AND CONSTITUTIONAL

Almost all of the cases show some reaction at the site of injection following the first inoculations, the reaction consisting of swelling, redness and itching, which last about twelve hours. After the third or fourth injection local reactions are practically absent even when 100 times the original dose is given. Any constitutional reaction bespeaks an overdose. Such reactions may consist in the production of an attack of hay fever or of a general pruritus with or without erythema or urticaria. In cases subject to asthmatic attacks with their disease, asthma likewise may be induced.

We have studied our cases from 1915 with reference to constitutional reactions. They can be summarized as follows:

TYPE	NUMBER CASES	TOTAL INJECTIONS	AVERAGING NUMBER INJECTIONS	NUMBER CASES WITH CONSTITU- TIONAL REACTION	TOTAL NUMBER OF REACTIONS
Early h. f.	98	1200	12+	25=24%	43=3.6%
Late h. f.	241	2992	12.4	74=31%	118=3.9%

In only one of the cases was the reaction sufficiently severe to cause apprehension. In this instance there was marked weakness and prostration with a rather rapid heart and a very low tension pulse. Recovery followed in the course of a few hours. As far as the effect of such reactions is concerned upon the general results of the protective inoculations we can say that apparently it tends to decrease the susceptibility to the protein and is therefore ultimately advantageous. In none of our cases, some of them having received treatment for three and four years, have we seen any evidence of any constitutional disturbance that could conceivably be traced to the inoculations. Longcope (18) and Boughton (20) have experimentally produced lesions in the kidneys of animals by repeated injections of heterologous protein, but this has usually been when large doses were given and anaphylactic shock was induced.

IV. RESULTS OF ACTIVE IMMUNIZATION

In 1914 Freeman reported on 84 cases of early hay fever treated by active immunization during the seasons of 1911-1912-1913. Freeman's results are summarized by him as follows:

For 34 seasons: Completely cured or insignificant symptoms.....	30.1 per cent
For 39 seasons: Hay fever greatly diminished.....	34.5 per cent
For 27 seasons: Hay fever diminished but slightly.....	23.9 per cent
For 13 seasons: Hay fever no better.....	11.5 per cent

In 1913 Clowes reported on 8 cases of late hay fever and states that "all the cases treated experienced a marked alleviation of general symptoms." Koessler treated 41 cases in four years. These can be summarized as follows:

Early hay fever, 5 cases	Prophylactic, 17	Completely free, 4 cases
Late hay fever, 36 cases	Phylactic, 19	Markedly free, 29 cases
		Not improved, 8 cases

Oppenheimer (13) treated 11 cases in 1914, 6 prophylactically and 5 phylactically. Of those prophylactically treated 1 was free of symptoms, 4 were markedly improved and 1 not im-

proved. Of the 5 phylactic cases 4 were markedly improved and one not relieved.

Ulrich treated 12 cases with "pollen toxin" in 1913. Nine showed satisfactory improvement.

The term "cure" is incorrect and misleading and should not be used in any discussion of results. Freeman's classification is very satisfactory and forms a valuable basis of comparison. The results in our own cases, covering the years 1912-1915 inclusive are listed in table 11 and summarized in table 12. By "greatly diminished" hay fever we mean an absence of 75 per cent of the usual amount of trouble.

This summary speaks for itself. The clinical results have been surprisingly good. If we consider the 3+ and 2+ classes together we find that satisfactory protection is offered to the early hay fever subjects in about 90 per cent whether treated phylactically or prophylactically.

Immunization in the late hay fever cases is somewhat more difficult. It is probably to be explained either by a greater toxicity of the protein or a larger amount of pollen in the air. It therefore requires a higher degree of immunity to protect.

V. DURATION OF IMMUNITY

Reasoning by analogy from the work in animal anaphylaxis we should not expect the protection afforded by active immunization in hay fever to be in any sense permanent. Freeman states however that "when a patient has been inoculated for one year with complete success, he has in the next year complete or almost complete immunity, but that in the third year he has but slight immunity left." Clowes tested 5 of his cases for their ophthalmic resistance five months after injections were stopped and in all cases it had dropped back to the point where it was before treatment. Koessler reports two cases that remained free for two years and 2 cases free for one year after receiving one series of injections. We have four such instances of apparent cure among our cases. But the reason for this is not clear and these results certainly do not hold for any considerable number of cases. We cannot therefore safely attribute their immune

TABLE II

Treatment of hay fever by active immunization. List of cases by year and according to results

A. Early hay fever

YEAR	PROPHYLACTIC				PHYLACTIC			
	+++	++	+	-	+++	++	+	-
1912						102		
1913	102	115 18, 29, 61, 112 115, 116	28, 111			37, 44, 46, 100 101, 103, 104, 106 107, 114	17, 108, 109, 110 116.	
1914	96	18, 27, 44, 61 65, 90, 100, 103 107, 115, 116, 131 145, 146, 147, 148 151, 153, 154, 159	53, 106, 109, 110 112, 143, 144, 150 155, 156			28, 29, 46, 158 161, 162, 163, 166 169, 172, 175, 178 179, 180, 182, 183 184, 188, 196, 197 200, 203, 327	108, 171, 185, 191 192, 194	111, 157, 173, 174
1915	44, 46, 50, 61 96, 109, 115, 146 153, 162, 163, 169 171, 172, 182, 183 184, 185, 194, 307 312, 314, 316, 319 322, 327, 330, 331 333, 334, 340, 344 353, 357, 368	53, 65, 90, 100 103, 106, 107, 112 147, 154, 155, 156 178, 188, 192, 200 306, 308, 318, 325 326, 341, 343, 348 352, 355, 356, 363 365	111, 151, 116, 166 293, 332, 346, 354 361	143, 175	371, 376, 384, 388 405, 411, 419, 436 438	6, 131, 378, 297 387, 389, 391, 394 396, 401, 408, 435 437, 452	372, 373	

B. Late hay fever

YEAR	PROPHYLACTIC				PHYLACTIC			
	+++	++	+	-	+++	++	+	-
1912						56, 114		
1913	12, 46	41, 62, 84 13, 18, 22, 29 31, 41, 43, 44 54, 57, 58, 59 61, 62, 63, 64 67, 68, 69, 74 75, 76, 80, 84 87, 93	14, 15, 24, 27 28, 30, 37, 55 56, 71, 72, 81 114, 176	126, 140 16, 36, 40, 42 47, 52, 66, 77	131	3, 6, 10, 20 21, 33, 38, 39 53, 70, 79, 88 92	71, 118, 125 32, 73, 78A, 78B 82, 85	66 25, 34, 86, 91

1014	46, 196	3, 13, 18, 19 20, 22, 27, 28 29, 39, 41, 43 44, 53, 57, 59 61, 63, 70, 76 79, 84, 93, 131 149, 169, 181, 199 203, 205, 210, 214; 218, 221, 226, 230 239, 605	12, 14, 15, 26 30, 31, 52, 54 64, 81, 85, 87 88, 92, 98, 145 148, 164, 167, 176 211, 212, 215, 216 219, 224, 225, 227 229, 231, 232, 233	32, 42, 58, 72 80, 86, 144, 201 202, 204, 206, 208 209, 213, 217, 220 223, 236	258, 262	6, 71, 234, 238 242, 244, 251, 252 255, 255, 256, 257 261, 263, 268, 274 279, 280, 281, 284 285, 286, 287	198, 228, 240, 241 243, 245, 246, 248 260, 264, 267, 275 276	247, 250, 254, 270 278
1015	32, 41, 44, 46 50, 61, 63, 64 70, 118, 169, 176 214, 230, 255, 368 375, 376, 381, 384 401, 406, 407, 411 414, 416, 423, 436 440, 444, 448, 451 457, 465, 467, 477 485, 500	3, 6, 12, 14 20, 21, 22, 26 35, 39, 43, 52 56, 57, 67, 69 76, 79, 81, 84 98, 131, 148, 149 167, 187, 190, 203 205, 226, 229, 232 234, 238, 239, 253 262, 263, 267, 273 276, 280, 283, 286 287, 306, 308, 321 328, 337, 338, 355 356, 364, 374, 377 382, 383, 386, 393 395, 399, 400, 403 404, 408, 409, 410 413, 415, 421, 422 425, 427, 428, 429 430, 432, 434, 435 437, 441, 443, 446 450, 453, 454, 461 462, 466, 468, 470 474, 475, 479, 481 482, 487, 488, 489 490, 493, 494, 495 496, 497, 498, 499 605	1, 31, 72, 73 92, 199, 211, 240 243, 257, 272, 302 304, 313, 319, 347 354, 358, 360, 365 366, 412, 420, 426 433, 445, 447, 449 458, 473, 478, 483 492	58, 93, 201, 275 312, 351, 357, 371 431	215, 506, 507, 509 510, 511, 512, 521 534, 550, 554, 567 570	13, 71, 83, 88, 285 297, 344, 505, 514 519, 520, 524, 527 528, 529, 531, 535 537, 543, 544, 549 551, 558, 568, 569 574, 577, 578, 579 583, 584, 586, 606	504, 508, 516, 517 532, 536, 539, 546 553, 555, 559, 560 566	519, 552, 581

+++ Hay fever absent or insignificant.

++ Hay fever greatly diminished.

+ Hay fever slightly diminished.

— Hay fever as bad as usual.

TABLE 12
SUMMARY OF TABLE 11
Showing the results of treatment by active immunization

A. Early hay fever

YEAR	TOTAL CASES	PROPHYLACTIC					PHYLACTIC				
		Number of Cases	+++	++	+	-	Number of Cases	+++	++	+	-
1912	2	1	0	100	0	0	1	0	100	0	0
1913	24	9	11	67	22	0	15	0	67	33	0
1914	64	31	3	65	32	0	33	0	70	18	12
1915	100	75	47	38	12	3	25	36	56	8	0

B. Late hay fever

YEAR	TOTAL CASES	PROPHYLACTIC					PHYLACTIC				
		Number of Cases	+++	++	+	-	Number of Cases	+++	++	+	-
1912	11	5	0	60	0	40	6	0	33	50	17
1913	74	50	4	52	28	16	24	4	55	25	16
1914	133	90	2	42	36	20	43	5	54	30	11
1915	251	189	20	57	18	5	62	21	53	21	5

+++ Hay fever absent or insignificant.

++ Hay fever greatly diminished.

+ Hay fever slightly diminished.

- Hay fever as bad as usual.

state entirely to their inoculations, for cases of hay fever do spontaneously become cured. We have 6 such instances of spontaneous cure among our records. In 2 of them, curiously enough, an early hay fever disappeared and was replaced by the late type. However, these cases as a result of their cure do not return to a normal state. They all possess definitely positive cutaneous reactions while an ophthalmic reaction is negative even to the strongest solution. A further study of these cases is being made in the hope that these natural results can be duplicated by artificial means.

In general, however, it is true that the cases satisfactorily immunized one season respond more readily to the treatment the ensuing year.

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Table of Abbreviations

Asth.....Asthma	Neg.....Negative.
A.....Aunt	Pat.Paternal
B.....Brother	Pro.....Prophylactic
B. Asth.....Bronchial asthma	Phyl.....Phylactic
E. H. F.....Early hay fever	Quin.....Quinine
F.....Father	R.....Ragweed
Gast. Ent....Gastro-enteritis	S.....Sister
G. M.....Grandmother	Straw.....Strawberries
G. F.....Grandfather	Sibs.....Sisters and Brothers
G. P.....Grass pollen	Sods.....Sons and Daughters
G.....Goldenrod	S. Fish.....Shell fish
H.....Husband	U.....Uncle
L. H. F.....Late hay fever	Unk.....Unknown
M.....Mother	Urtic.....Urticaria
Mat.....Maternal	W.....Wife

PROTOCOL OF THE CASES FORMING

CASE NO.	FAMILY HISTORY											
	SEX	AGE	Paternal		Maternal		Sibs normal		Sibs sensitized			Other
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
1	F.	45					5 Sis.					
2	M.	14										
3	M.	5	F. No. 455.	Gastro-enter. from egg and asth.								
4	F.											
5	M.	11			Urtic. straw.			B. No. 537. B. No. 543.	L. H. F. R. & G. L. H. F. R. & G.	17 13		
6	M.	45										
7	M.											
8	M.	30										
9	F.	50										4 Sodo. O.K. 16 to 23 yrs. 3 Sons. O.K. Oldest 12.
10	M.											
11	F.		G. M.	Asth.								
12	M.	54										Wife O.K. D. 131. D. OK. Age 27.
13	F.	32										
14	M.	39					B. OK.					
15	F.	58					None.		None.			1 S. \$177. 1 D. \$176. 3 sods. W's F. 1 D. 1 S.
16	M.	64	Neg.		Neg.		2 B. 1 S.					
17	F.	27	U.	E. and L. H. F.								
18	M.	40	Neg.		Neg.		1 S.		None.			4 chil. Oldest 7 yrs.
19	F.	31	Neg.		Neg.		None.		None.			
20	F.	32	Neg.		Neg.		2 B. 1 S.			None.		

THE BASIS OF THE PRESENT STUDY

PATIENT'S HISTORY												
latives	Age of onset	Diag.	Cause	Other sensit.	Cause	Results						
Diag.					Late or early	1912		1913		1914		1915
	26	Late H. F. with asth.	R and G.	Urticaria. Gastro-enter. H. F.	Clams, Salmon. Orris.	L.					Pro.	+
	12	Asthma.										
	4	Late H. F. E. H. F.	R. R.	Urtic.	Egg.	L.		Phy.	++	Pro.	++	Pro. ++
	8	Irreg. H. F. Asth.	Mullen and Timothy. Daisy and Horse.	Asth. and H. F.	Horse							
	42	L. H. F.				L.		Phy.	++	Phy.	++	Pro. ++
	44	E. H. F.				E.					Phy.	++
	27	L. H. F.	R. and G.									
	38	Asth.	Rye.									
	10	L. H. E.	R. and G.									
	28	Asth.	Corn. Privet. Chestnut. Clematis. R.									
	31	L. H. F. with asth.				L.		Pro.	+++	Pro.	+	Pro. ++
E. and L. H. F.	12	L. H. F.	Ragweed Astor. Redtop.	Erythema.	Straw.	L.		Pro.	++	Pro.	++	Phy. ++
		E. H. F.		Angio N. edema.	Crab.							
	35	L. H. F.	R. and G.			L.		Pro.	+	Pro.	+	Pro. ++
H. F. with asth.	38	L. H. F.	R.	None.		L.		Pro.	+	Pro.	+	
L. H. F. OK.												
L. H. F. Urtic. OK.	34	L. H. F.	R.	None.		L.		Pro.	(-)			
	23	E. H. F.	Corn Pr.	None		E.		Phy.	+			
OK.	16	E. H. F. L. H. F. Asth.	G. P. R. G.	Edema. Asth.	Quin. Horse.	E.		Pro.	++	Pro.	++	
						L.		Pro.	++	Pro.	++	
	24	L. H. F.	R and G.	None.		L.		Phy.	++	Pro.	++	
	25	L. H. F.	R. and G.	Urtic.	Quin.	L.		Phy.	++	Pro.	++	Pro. ++

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Other
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
21	F.	36	Neg.	Neg.	1 S.	..	1 S.	H. F.
22	M.	37	F.	Asth. Ecz.	G. M.	L. H. F. Asth.	1 S. 1 S. 1 S.	32 22	None.	2 chil.
23	F.	57	Neg.	Neg.	D. 28.
24	F.	42	Neg.	Neg.	B. S.	..	B. 1. S.	L. H. F. E. H. F.
25	F.	27	Neg.	Neg.
26	M.	36	Neg.	Neg.	S.	..	None.
27	M.	33
28	M.	37	Neg.	Neg.	C.
29	M.	25
30	F.	40	D. 13. S. 7.
31	F.	43	Neg.	M.	E. H. F. L. H. F. Urtic.	B.	46	None.	Hus. # 112. D. 10. S. # 385.
32	F.	23	Neg.	M.	Asth.	1 C. 1 C. # 115.
33	F.	42	Neg.	Neg.
34	M.	45	Neg.	Neg.
35	F.	33	Neg.	M. # 605. Gt. G. F. A. # 107.	L. H. F. Asth. E. H. F.	S 1.	38	None.
36	F.	63	Neg.	Neg.
37	F.	21	Aunt.	E. H. F. Asth.	Neg.
38	M.	54	Neg.	Neg.
39	F.	27	F. # 133.	B. asth.	Neg.	S.	..	None.	2 chil.
40	F.
41	M.	32	F.	L. H. F. w i t h asth.	Neg.	2 B. 1 S.	..	B.	E. H. F.
			G. M.	L. H. F. w i t h asth.
42	M.	36	F. G. F.	Asth. Asth.	Neg.	None.	..	None.

PATIENT'S HISTORY															
Relatives	Age of onset	Diag.	Cause	Other sensit.	Cause	Results									
						Late or early	1912		1913		1914		1915		
Diag.															
OK.	28	L. H. F.	R.	None.		L.			Phy.	++			Pro.	++	
	16	L. H. F.	R and G.	None.		L.			Pro.	++	Pro.	++	Pro.	++	
OK.	50	B. Asth.	Unkn.												
	9	L. H. F.	R. G.	Urtic.	Oatm.	L.			Pro.	+					
				Urtic.	Fish.										
	7	L. H. F., E. H. F.	R. G.	Urtic.	Fish.	L.			Phy.	(-)					
	26	L. H. F.	R. G.	None.		L.					Pro.	+	Pro.	++	
	29	L. H. F., E. H. F., Asth.	R. G. P	None.		L. E.			Pro.	+	Pro.	++			
E. H. F.	33	L. H. F., E. H. F.	R. G. G. P.	None.		L. E.			Pro.	+	Pro.	++			
									Pro.	+	Phy.	++			
	5	L. H. F., E. H. F., Asth.	R. G. G. P.	H. F. Urtic.	Celery.	L. E.			Pro.	++	Pro.	++			
									Pro.	++	Phy.	++			
E. H. F.	32	L. H. F.	R. G.	None.		L.			Pro.	+	Pro.	+			
OK.		Asth.													
E. H. F.	17	L. H. F.	R. G.	Urtic.	Straw.	L.			Pro.	++	Pro.	+	Pro.	+	
OK.		with asth.													
E. H. F.		E. H. F.													
L. H. F., with asth.															
L. H. F.	5	L. H. F. with asth.	R. G.	H. F.	Horse.	L.			Phy.	+	Pro.	(-)	Pro.	+++	
E. H. F.															
	32	L. H. F. E. H. F. cured 5 yrs.	R. G.	None.		L.			Phy.	++					
	42	L. H. F.	R. G.	Gastro-En.	Lob.	L.			Phy.	(-)					
	3	B. Asth.		Urtic.	Fish.								Pro.	++	
	53	L. H. F. Asth.	R.	None.		L.			Pro.	(-)					
	7	L. H. F. E. H. F.	R. G. P.	Urtic. Urtic.	Straw. Sh. Fish.	L. E.			Pro. Phy.	+					
									Phy.	++					
OK.	49	L. H. F.	R.	None.		L.			Phy.	++					
	24	L. H. F.	R.	None.		L.			Phy.	++	Pro.	++	Pro.	++	
		L. H. F.	R.			L.			Pro.	(-)					
	15	L. H. F.	R. G.			L.	Pro.	++	Pro.	++	Pro.	++	Pro.	+++	
	22	L. H. F. with Asth.	R.	None.		L.			Pro.	(-)	Pro.	(-)			

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Othe
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
43	F.	36	A.	Asth.	Neg.	None.	..	None.
44	F.	26	Neg.	Neg.	S.	34	None.
							B.	31				
							B.	28				
45	F.	37	G. M.	Asth.	B.	L. H. F.	..	S. 11.
												S. 9.
												D. 6.
46	M.	42	Neg.	Neg.	None.	..	None.	W.
47	M.	33	Neg.	A.	E. H. F.	B.	..	2 B.	L. H. F.	..	4 chil.
							S.					
48	M.	44
49	M.	63
50	M.	43	Neg.	M. #124.	H. F. with asth.	None.	..	None.	W.
												W.'s Sis.
												Child.
												Child.
51	F.	15	S.	E. H. F.
52	F.	30	Neg.	Neg.
53	M.	40	Neg.	Neg.
54	M.	45	Neg.	Neg.
55	F.	25	Neg.	Neg.
56	M.	29
57	M.	34	Neg.	Gt. Aunt. M. #151. G.-Mother.	L. H. F. E. H. F. E. and L. H. F.	Sis. 109.	E. H. F. fr. G. pollen.	32
58	M.	34
59	M.	34
60	F.	39	Father.	Bron.- Asth.	Neg.	Bro. Sis.	28 37	Bro. Sis.	Asthma- Horse. L. H. F.- Ragw. H. F. = Orris. Urtic. = Straw.	35 31	Cousin.

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Other
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
61	F.	48	Neg.	Neg.	Sis.	50	None.
62	M.	14	Neg.	Uncle.	L. H. F. w i t h asthma.	Sister.	11
63	F.	20	Father.	E. H. F. L. H. F.	Aunt. Neg.	2 Bros.	..	None.
64	M.	37	Neg.	Neg.	Sis. 5 Bros.
65	M.	14	Uncle.	L. H. F.	Neg.
66	M.	42	Neg.	Neg.	Wife and her fam- ily neg. 1 child 12. 1 child 9. 1 child 6.
67	F.	38	Neg.	G.-mother.	E. H. F.	Sister.	Urtic. from Straw.	Husb. sister 3 child. OK.
68	M.	33	Neg.	Neg.	2 Bros.	None.
69	F.	43	Neg.	Neg.	Husb. OK. 2 sons OK.
70	F.	37	Neg.	G.-father. Gt.-Uncle.	L. H. F. L. H. F.	1 Bro.	1 Bro.	Asth. = Unk.
71	M.	38	Neg.	Neg.
72	M.	29	Father.	A c u t e Gastr., Straw.	Mother.	Urtic. = Unk.	None.	Sister.	Asth. = Unk.	33
			Uncle.	Asth. = Unk.	Sister.	Urtic. = fish.	26
			Aunt.	Asth. = Unk.
73	F.	36	Father.	Asth. = Unk.	Mother.	L. H. F.	1 Sister.	40	Sister.	L. H. F.	32	Nephew (son of sister of 40).
74	M.	31	Neg.	Neg.	1 Sister.
75	M.	44	Neg.	Neg.	1 Bro.	L. H. F.
76	M.	31	Mother.	E. and L. H. F.	1 each Sib.	None.

PATIENT'S HISTORY														
relatives	Age of onset	Diag.	Cause	Other sensit.	Cause	Results								
						Late or early	1912		1913		1914		1915	
	25	L. H. F.	R.	Asth.	Horse.	L.	Pro.	++	Pro.	++	Pro.	+++
		E. H. F.	G. pollen.	H. F.	Horse.	E.	Pro.	++	Pro.	++	Pro.	+++
	3	L. H. F. with asthma.	R. and G.			L.	Pro.	++	Pro.	++				
	6	L. H. F.	R. and G.			L.	Pro.	++	Pro.	++	Pro.	+++
	26	L. H. F.	R.			L.	Pro.	++	Pro.	+	Pro.	+++
	5	E. H. F.	G. pollen.	H. F.	Cat.	E.			Pro.	++	Pro.	++
	5	L. H. F.	R.			L.	Phy.	-	Pro.	-				
E. H. F. OK.														
L. H. F.														
E. H. F.	35	L. H. F. with asth.	R.	Gastro- ent. Urtic.	Straw. Straw.	L.	Pro.	++			Pro.	++
	31	E. H. F.	G. pollen. (Cured.)											
	21	L. H. F.	R.	None.		L.	Pro.	++				
23 and 20.	23	L. H. F.	R.	None.		L.	Pro.	++			Pro.	++
	4	E. H. F.	Cured 10 yrs.											
		L. H. F.	R. and G.	None.		L.	Phy.	++	Pro.	++	Pro.	+++
	24	E. H. F. L. H. F. with asth.	G. pollen. R. and G.			L.	Phy.	+	Pro.	+	Phy.	++	Phy.	++
	16	E. H. F.	G. pollen.	Edema.	Fish.	L.	Pro.	+	Pro.	-	Pro.	+
		L. H. F. with asth.	R. and G'rod.	Urtic. Gastro- ent.	Celery. Egg.									
L. H. F.	21	L. H. F.	R.	None.		L.	Phy.	+			Pro.	+
	3	L. H. F.	R.	None.		L.	Pro.	++				
	14	E. H. F. L. H. F. w. asth.	R.	None.		L.	Pro.	++				
	17	E. H. F. L. H. F.		Gastro- ent.	Fish.	L.	Pro.	++	Pro.	++	Pro.	++

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Other
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
77	M.	50	Neg.	Mother.	Asth.— Unk.	Sister	L. H. F.
					G.-father.	Asth.— Unk.				
78a	F.	20	Aunt.	L. H. F.— R. and G.	Neg.	1 Sister.	H. F.— Horse.	12
									1 Sister.	L. H. F.— R. and G.	17 (78b.)
78b	F.	17	Aunt.	L. H. F.— R. and G.	Neg.	1 Sister.	H. F.— Horse.	12
									1 Sister.	L. H. F.— R. and G.	20 (78a.)
79	M.	44	Neg.	Neg.
80	F.	36	G.-father.	Asth.— Unk.	Neg.	3 Bros.			
			Father.	Asth.— Unk.			6 Sis.			
81	M.	30	Neg.	Neg.				Cousin.
82	F.	30	Neg.	Mother.	L. H. F.
83	M.	30	Neg.	Neg.	5 Bros. 1 Sis.			
84	M.	30	Neg.	Neg.	5 Bros. 2 Sisters.			
85	M.	20	Neg.	Neg.	1 Bro.	L. H. F.	13
86	M.	48	Neg.	Neg.	1 Brother. 3 Sisters.			Neg.
87	F.	25	Neg.	Neg.				Husb. O. Child 2½
88	M.	53	Neg.	Neg.	1 Bro.				Wife's fa 2 child 25-26.
89	F.	41	Neg.	Neg.
90	F.	36	Father.	Asth.	Neg.	8 Sibs.	None.			Pat. Cou in.
91	M.	54	Grand-f.	Asth.— Unk.	1 Bro.	Asth.— Unk.

PATIENT'S HISTORY														
Patient's Diag.	Age of onset	Diag.	Cause	Other sensit.	Cause	Late or early	RESULTS							
							1912		1913		1914		1915	
	30	L. H. F. with Asth.	R.	None.		L.			Pro.	-				
	15	L. H. F.	R. and G.	Edema.	Fish.	L.			Phy.	+				
	11	L. H. F.	R. and G.			L.			Phy.	+				
	20	L. H. F.	R. and G.	None.		L.			Phy.	++	Pro.	++	Pro.	++
	16	E. H. F.												
		L. H. F. with Asth.	R. and G.			L.			Pro.	++	Pro.	-		
H. F.	21	L. H. F. with Asth.	R.											
	15	L. H. F.	R.			L.			Phy.	+				
	28	L. H. F.	R. and G.	Gastro-ent.	Crabs.	L.							Phy.	++
	20	L. H. F. with asth.	R.			L.	Pro.	++	Pro.	++	Pro.	++	Pro.	++
	10	E. H. F.	G. pollen.											
		L. H. F.	R. and G.			L.			Phy.	+	Pro.	+		
	36	L. H. F.	R.	None.		L.			Phy.	-	Pro.	-		
	8	L. H. F. with Asth.	R. and G.			L.			Pro.	++	Pro.	+		
H. F.		E. H. F.												
ak. K.	28	L. H. F. formerly with Asth.	R. and G.	None.		L.			Phy.	++	Pro.	+	Phyl.	++
	39	L. H. F. with Asth.	Not tested.	None.										
		E. H. F.												
th.	26	E. H. F. with Asth.	G.-pollen.	None.		E.					Pro.	++	Pro.	++
		L. H. F.												
	39	L. H. F. with Asth.				L.			Phy.	-				

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Other
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
92	F.	40	Neg.	Neg.	2 Pat. Cousins.
93	M.	38	Neg.	Mother.	L. H. F.	4 Bros. 1 Sis.	None.
94	M.
95	M.
96	M.	30	Neg.	Neg.	1 Sis. #218.	L. H. F. Urtic.— Fish.	32	Pat. 1st. C. 196. Wife's f. 2 Child.
97	M.
98	F.	24	Neg.	Neg.	Bro. Sister.	30 18	Mat. 3rd C.
99	F.	24	Neg.	Neg.
100	M.	44	1st C.
101	M.	17	Neg.	Neg.
102	F.
103	F.	33	Neg.	Grand-M.	Asth.	1 Bro.	L. H. F.— Asth.	Mat. 1st C
104	F.	38	Neg.	Grand-M.	Bron.— Asth.	None.	1 Bro. 1 Bro.	E. H. F. L. H. F.	40 35	Pat. C. 1 child 8. 1 child.
105	F.	12	Bro.	42
106	F.	33	Father.	L. H. F.	Mother.	Asth.— Unk.	Bro. Bro.	40 28	1 Sister	Urtic.— Unk.	38
107	F.	60	Neg.	Grand-F.	Asth.— Unk.	Sister #605	L. H. F.	56	Niece No. 45.
108	F.	35	Mother.	H. F. and asth.
109	F.	32	Mother #151. Grand-M. Great-Aunt.	E. H. F. E. and L. H. F. L. H. F.	Bro. No. 57.	L. H. F., R. and G.	34
110	M.	36	Neg.	Neg.	None.
111	M.	34	Neg.	Neg.	None.	Neg.
112	M.	38	Neg.	Neg.	Wife #3. Son #385. Daughter
113	F.
114	M.	27	Father.	Asth.	Neg.	Unk.

PATIENT'S HISTORY															
relatives	Age of onset	Diag.	Cause	Other sensit.	Cause	Results									
						Late or early	1912		1913		1914		1915		
H. F.	29	L. H. F. with Asth.	R. and G.			L.			Phy.	++	Pro.	+	Pro.	+	
	10	L. H. F. with Asth.	R. and G.	None.		L.			Pro.	++	Pro.	++	Pro.	-	
Asth.	23	E. H. F.	G. pollen.	None.		E.					Pro.	+++	Pro.	+++	
Asth. OK.															
*59.	9	L. H. F. with Asth.	R.	None.		L.					Pro.	+	Pro.	++	
L. H. F.															
H. F.	20			H. F.	Horse.										
H. F.	30	E. H. F. with Asth.				E.			Phy.	++	Pro.	++	Pro.	++	
	14	E. H. F.	G. pollen.	Urtic.	Unk.	E.			Phy.	++					
For 6 yrs.															
H. F. and Asth.	30	E. H. F. ♂ Asth.	G. pollen.	None.		E.	Phy.	++	Pro.	+++	Pro.	++	Pro.	++	
L. H. F.	18	E. H. F.	Not tested.	Urtic. H. F.	Straw. Horse.	E.			Phy.	++					
Asth. H. F.															
	11	Bron.—Asth.	Unk.												
	25	E. H. F.	G. pollen.	Gastroent.	Cucumber.	E.			Phy.	++	Pro.	+	Pro.	++	
Asth.	20	E. H. F.	G. pollen.			E.			Phy.	++	Pro.	++	Pro.	++	
	31	E. H. F.	G. pollen.	H. F.	Orris.	E.			Phy.	+	Phy.	+			
	30	E. H. F.	G. pollen.			E.			Phy.	+	Pro.	+	Pro.	+++	
	33	E. H. F.	G. pollen.	Urtic.	Berries.	E.			Phy.	+	Pro.	+			
	28	E. H. F.	G. pollen.	None.		E.			Pro.	++	Phy.	+	Pro.	+	
L. H. F.	24	E. H. F.	G. pollen.			E.			Pro.	++	Pro.	+	Pro.	++	
E. and L. H. F. OK.															
	7	L. H. F. with Asth. Bron.—Asth. E. H. F.	R. and G.			E.			Phy.	++					
						L.	Phy.	++	Pro.	+					

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Other
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
115	M.	32	Neg.	Neg.	Brother. Brother.	39 34	1 Bro.	L. H. F.	15	Wife's fam. 1 child 13. Cousin *32.
116	F.	34	Aunt.	Asth.— Unk.	Uncle. Aunt.	Asth.— Unk. Asth.— Unk.	1 Bro. 1 Sis. 1 Sis.	32 37	None.		Cousin.
117	F.	45	31	Unk.		Daugh. Daugh.
118	M.	46	Neg.	Neg.	1 Sis.	44		Wife. Son. 17.
119	M.	22	Neg.	Neg.	2 Bros. 1 Sister.	None.		
120	F.	36	Neg.	Neg.	6 Sibs.	None.		
121	M.	18	Neg.	Mother.	Urtic.— Unk.	2 Sis. 1 Bro.	None.		
122	F.	50	Neg.	Mother.	L. H. F.		
123	M.	45		
124	F.	74	Neg.	Neg.	1 Bro.		Husb. Son *50.
125	F.	50	Father.	L. H. F.	Neg.		Unk.
126	M.	35
127	M.	20	Neg.	Mother.	L. H. F.	2 Sis. 1 Bro.	None.		Cousin..
128	M.	48	Neg.	Neg.		Unk.
129	M.	46	Aunt.	E. H. F.	Neg.	1 Bro. 1 Sis. 1 Sis. 1 Sis.	50 44 42 36	1 Sis. *457.	L. H. F. & Asth. and Bron.— Asth.	29	Neg.
130	F.
131	F.	26	Father.	L. H. F. and Asth.	1 Sis.	27	None.
132	M.	26	Neg.	Neg.
133	M.	60		Daugh. *39.
134	F.	40	Grand-f	Asth.— Unk.	Mother.	L. H. F.	None.	None.		None.

[illegible]

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Other
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
135	F.	53	Neg.	Neg.						
136	F.								
137	F.								
138	F.		Neg.	Neg.						
139	F.	19							
140	M.	41							
141	M.	27							
142	F.								
143	F.	50	Neg.	Neg.	5 Sibs.		None.			
144	M.	42	Father.	L. H. F. and asth.	Neg.	Sister.	50	Sister	L. H. F. 3 Asth.	51	Pat. G. F. and G. M. Pat. 6 Unc. and Aunts.
			Uncle (twin).	L. H. F.							
145	M.	7	Neg.	Neg.						
146	M.	40	Neg.	Neg.	Bro.	37	None.			Wife.
				Sis.	33				3 child. { 13 10 8
147	F.	61	Neg.	Neg.						Husb's Sis 2 child. { 20 30 1 child 24.
148	M.	34		None.		None.			Pat. Cous- in.
149	F.	39	Neg.	Uncle.	E. H. F.	11 Sibs.		None.			
150	F.	31	Father.	E. H. F.	Neg.	1 Sis.		Bro.	E. H. F.		
151	F.	60	Neg.	Mother.	E. and L. H. F.					Mat. Cousin. Daugh. #109. Son #57.
				Aunt.	L. H. F.						
152	F.								
153	F.	26	Father.	L. H. F. E. H. F.	Neg.						
154	F.	29	Neg.	Neg.	2 Sis.					Husb. 2 child.
155	M.	18	Neg.	Mother. Uncle.	L. H. F. E. and L. H. F. Asthma.						
156	M.	44	Uncle.	L. H. F.	Grand-m.						Wife. 2 child. {

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Other
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
157	F.	45	Neg.	Neg.	Cousin. Husb.
158	F.	34	Neg.	Mother.	E. and L. H. F.	Sister.	Asth.= Animals.	2 Sons { 19 24 1 Son 21. Husb.'s Sis. Daugh. 8 Daugh. 6 yrs.
159	M.	7	Neg.	Neg.
160	F.	43	Aunt.	Asth.= Unk.	Neg.	1 each Sib.	None.
161	F.	45	Neg.	Neg.	3 Sibs.	1 Bro.	L. H. F.	Cousin.
162	F.	38	Neg.	Neg.	Neg.
163	M.	34	Neg.	Neg.	Bro.	E. H. F.	Nephew #602.
164	F.	40	Neg.	Neg.
165	F.	32	Neg.	Neg.
166	F.	69	Neg.	Uncle.	E. and L. H. F.	1 Bro.	Asth.— Unk.
167	M.	39	Great aunt.	L. H. F.	Neg.	Pat. 2nd cousin.	L. H. F.
168	F.	35	Father.	L. H. F. & asth.	Neg.
169	F.	24	Neg.	Gt. g. moth- er.	Asth. Urtic.— celery and to- mato. L. H. F.	2 Sibs.	21 & 17	None.
170	F.	70	Neg.	Aunt.
171	F.	31	Neg.	Neg.
172	F.	39	Aunt.	L. H. F.	Neg.	2 Bros.	1 Bro.	E. H. F. and Asth.
173	M.	34	Neg.	Neg.	1 Sis.	39	1 Bro.	L. H. F. Cured.	43	Wife's Mother. 3 { 2 mos chil. { 7 yrs. 4 yrs.

PATIENT'S HISTORY														
Initiatives	Age of onset	Diag.	Cause	Other sensit.	Cause	Late or early	Results							
							1912		1913		1914		1915	
E. H. F. Neg. OK.	15	E. H. F.	Grass-p.	None.		E.					Phy.	-		
E. H. F. H. F. E. and L. H. F. H. F.	27	E. H. F.	Grass-p.	Urtic. H. F.	Fish. Orris.	E.					Phy.	++		
	4	E. H. F.	Grass-p.			E.					Pro.	++		
	6	Irreg. H. F. & Asth. Bron. Asth. Pulm. Tb.	R. and G. p.	Gastro- ent.	Fish.									
E. H. F.	25	E. H. F. & Asth.	Grass-p.	None.		E.					Phy.	++		
	29	E. H. F.	Grass-p.			E.					Phy.	++	Pro.	+++
E. and L. H. F.	14	E. H. F.	Grass-p.	None.		E.					Phy.	++	Pro.	+++
	35	L. H. F.	R.			L.					Pro.	+		
	28	Vasomotor Rhinitis												
	57	E. H. F.	Grass-p. Rose-p.			E.					Phy.	++	Pro.	++
	12	L. H. F.	R. and G.			L.					Pro.	+	Pro.	++
	34	Bron. Asth.		Urtic. Urtic.	Fish Berries.									
	21	Asth. E. H. F. L. H. F.	R. and G. Grass-p.	Indiges- tion.	Fish, clams and straw.	E.					Phy.	++	Pro.	+++
				H. F. and Asth. H. F. and Asth.	Horse. Dog- cat.	L.					Pro.	++	Pro.	+++
	59	E. H. F.	Neg. to pollen.	None.										
	15	L. H. F. } & Asth. } E. H. F. } & Asth. }	G. pollen.			E.					Phy.	+	Pro.	+++
	17	E. H. F. & Asth.	Grass-p.	None.		E.					Phy.	++	Pro.	+++
sth.	26	E. H. F.	Grass-p.			E.					Phy.	-	Pro.	No re- port
OK.														

PATIENT'S HISTORY														
Patient's Name	Age of onset	Diag.	Cause	Other sensit.	Cause	Results								
						Late or early	1912		1913		1914		1915	
H. F. K.	10	E. H. F.	Grass-p.			E.					Phy.	-		
		E. H. F.	Grass-p.			E.					Phy.	++	Pro.	-
		E. Asth.												
	5	L. H. F.	R.	H. F. and Asth.	Horse.	L.			Pro.	+	Pro.	+	Pro.	+++
		E. Asth.												
		E. H. F.												
	5	Asth.	R. and G. corn and grass pollen.											
H. F. K.	24	E. H. F.	Grass pollen.			E.					Phy.	++	Pro.	++
		E. Asth.												
Horse asth.	13	E. H. F.	Grass-p.			E.					Phy.	++	Phy.	
		E. Asth.												
		L. H. F.	R. and G.											
	18	E. Asth.												
		E. H. F.	Grass-p.	None.		E.					Phy.	++		
		L. and H. F.	R. and G.	None.		L.					Pro.	++	Pro.	
	17	E. H. F.												
		E. H. F.	Grass-p.	Urtic.= Straw.		E.					Phy.	++	Pro.	+++
	20	E. H. F.	Grass-p.	Urtic.= Unk.		E.					Phy.	++	Pro.	+++
		E. asth.		Ang. N. Ed.= Un-known.										
K.	17	E. H. F.	Grass-p.	None.		E.					Phy.	++	Pro.	+++
		E. Asth.												
		E. H. F.	Grass-p.	None.		E.					Phy.	+	Pro.	+++
	4	Bron.-asth	(Pollen neg.)											
	3	E. H. F.	Grass-p.			L.							Pro.	++
		L. H. F.	R. and G.											
	15	E. H. F.	Grass-p.	None.		E.					Phy.	++	Pro.	++
		E. Asth.												
		Irreg. H. F.												
	22	L. H. F.	Pollen-neg.											
		E. H. F.												
		E. H. F.	Grass-p.	None.		E.					Phy.	+		
H. F. K.	28	E. H. F.												

CASE NO.	SEX	AGE	FAMILY HISTORY										
			Paternal		Maternal		Sibs normal		Sibs sensitized			Oth	
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative	
192	M.	36											
193	F.	30	Neg.		Neg.								
194	M.	37	Neg.		Neg.								Son. #3:
195	M.	35											Cousin #96.
													Cousin #218.
196	F.	32	Neg.		Neg.								
197	F.	40											
198	M.	60	Neg.		Neg.		6 Sibs.		None.				1 child.
199	M.	11	Neg.		Grand-M. Gt. grand aunt. Mother. Aunt. 2 Uncles.	L. H. F. L. H. F. & Asth. Urtic— Unk. E. and L. H. F. dog and horse. L. H. F.							1 child.
200	F.												
201	F.	32	Neg.		Neg.		3 Sibs.		None.				
202	M.	47	Neg.		Neg.		2 Sibs.		None.				Wife's fa
													2 child.
203	M.	16	Father. Gd. father.	E. and L. H. F. Asth.	Neg.		1 Bro.	19	None.				Half-sist #153.
204	M.	35	Neg.		Neg.								
205	M.	15	Aunt.	E. H. F.	Neg.								
206	F.	17											
207	F.	36	Father.	Asth.	Neg.		1 Sister.		None.				
208	F.	55	Neg.		Neg.								
209	F.	38	Neg.		Neg.		2 Sibs.		None.				
210	M.	54	Neg.		Mother.	L. H. F. & Asth	2 Sis.		2 Bros. 1 Sis.	L. H. F. (cured) E. H. F.			

PATIENT'S HISTORY															
Patient	Diag.	Age of onset	Diag.	Cause	Other sensit.	Cause	Late or early	Results							
								1912		1913		1914		1915	
		30	E. H. F.	Grass-p.	Urtic.	Crabs.	E.					Phy.	+	Pro.	++
		18	E. H. F.		None.										
H. F.		14	E. H. F.	Grass-p.	None.		E.					Phy.	+	Pro.	+++
H. F.		7			H. F. and Asth.	Horse.									
H. F.		7	E. H. F.	Grass-p.	None.		E.					Phy.	++		
			L. H. F.	R.			L.					Pro.	+++		
		25	E. H. F.	Grass-p.			E.					Phy.	++		
			ē Asth.												
H. F.		14	L. H. F.	R. and G.	None.		L.					Phy.	+	Pro.	++
			ē Asth.												
		3	L. H. F.	R. and G.	None.		L.					Pro.	++	Pro.	+
			E. H. F.				E.					Phy.	++	Pro.	++
		26	L. H. F.				L.					Pro.	-	Pro.	-
			ē Asth.	R.											
			E. H. F.	Neg to grass-p.											
		17	L. H. F.	R. and G.	Ang. N. Ed. ē Asth.	Fish.	L.					Pro.	-		
			ē Asth.												
			E. H. F.												
H. F.		4	E. H. F.	Grass-p.	None.		E.					Phy.	++	(cured?)	
			L. H. F.	R. and G.			L.					Pro.	++	Pro.	++
		34	L. H. F.	R. and G.	Urtic.	Straw.	L.					Pro.	-		
		7	L. H. F.	R. and G.	Urtic.	Shell fish and straw.	L.					Pro.	++	Pro.	++
		8	L. H. F.	R.			L.					Pro.	-		
		28	Asth.	Neg. to pollen.											
		54	L. H. F.	R.	None.		L.					Pro.	-		
		14	L. H. F.	R.	Urtic.	Unk.	L.					Pro.	-		
			ē Asth.		H. F.	Perfumes and sachet.									
		50	L. H. F.	R. and G.	Ang. n. edema.	Unk.	L.					Pro.	++		

[illegible]

PATIENT'S HISTORY														
Patient	Age of onset	Diag.	Cause	Other sensit.	Cause	Results								
						Late or early	1912		1913		1914		1915	
H. F.	14	L. H. F. ̄ Asth.	R. and G.	Asth.	Horse.	L.	Pro.	+	Pro.	+
tic.- Oatmeal. K.	32	L. H. F. ̄ Asth. E. H. F. ̄ Asth.	R.	None.	L.
.....	25	L. H. F.	R.	Urtic.	Unk.	L.	Pro.	-
H. F.	L. H. F. ̄ Asth. E. H. F.	R. and G.	L.	Pro.	++	Pro.	+++
81.	23	L. H. F. ̄ Asth. E. H. F.	R.	L.	Pro.	+	Phy.	+++
thma.- Horse.	19	L. H. F. E. H. F.	R. and G.	Urtic.	Straw and peach- es. Horse.	L.	Pro.	+
.....	2	L. H. F. ̄ Asth.	R. and G.	None.	L.	Pro.	-
.....	24	L. H. F.	R. and G.	Erythema.	Shell Fish.	L.	Pro.	++
.....	39	L. H. F. ̄ Asth. E. H. F.	R.	L.	Pro.	+
.....	11	L. H. F. ̄ Asth.	R.	None.	L.	Pro.	-
.....	3	L. H. F.	R. and G.	None.	L.	Pro.	++
K. K.	28	L. H. F. ̄ Asth.	R.	None.	L.	Pro.	Pro.
.....	42	L. H. F.	R. and G.	None.	L.	Pro.	-
.....	19	L. H. F.	R. and G.	None.	L.	Pro.	+
.....	19	E. H. F.	R. and G.	None.	L.	Pro.	+
.....	32	L. H. F.	R.	None.	L.	Pro.	++	Pro.	++
.....	33	L. H. F. ̄ Asth.	L.	Pro.	+
.....	14	L. H. F. E. H. F.	R. and G.	None.	L.	Phy.	+	Phy.
and L. H. F.	19	L. H. F.	R. and G.	Sore- throat.	Straw.	L.	Pro.	+	Pro.	++
K.	8	E. H. F. L. H. F. L. H. F.	G.-p. R. and G. Not tested.	None.	L.	Pro.	++	Pro.	+++

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Othe
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
232	M.	25	Neg.	Neg.	None.	..	None.	
233	M.	31	Neg.	Neg. Grand-M.	L. H. F. & Asth.	
234	F.	35	Aunt.	Asth.	Neg.	
235	M.	30	
236	F.	33	Father.	L. H. F. & Asth.	Neg.	1 Bro.	Urtic. Straw.	Husb. fa ther.
								..	1 Bro.	Urtic. straw and egg plant.	Husband.
								1 child 1½
237	F.	21	Neg.	Neg.	
238	M.	35	Neg.	Neg.	None.	..	None.	1 child 11
239	F.	36	Neg.	Neg.	
240	M.	33	Neg.	Neg.	Bro.	L. H. F.	
241	F.	36	Neg.	Neg.	2 Sibs.	..	None.	
242	F.	54	Neg. (Switzerland).	Neg. (Switzerland).	None.	None.	..	4 child.
243	F.	76	Father.	Asth.	Neg.	5 Sibs.	None.	..	2 child.
244	F.	25	Neg.	Neg.	
245	F.	29	Neg.	Neg.	Sister.	L. H. F.	
246	F.	23	Neg.	Neg.	3 Sibs.	..	None.	
247	F.	35	Neg.	Neg.	1 Sis.	..	None.	Pat. Cou in. Husb. fa 3 child. }
248	M.	29	Father.	E. H. F.	Neg.	4 Sibs.	None.	..	Pat. Cou in. Wife's fa 2 child.
249	M.	20	Neg.	Neg.	
250	M.	37	Neg.	Neg.	
251	M.	42	Neg.	Neg.	
252	F.	36	Neg.	Neg.	

PATIENT'S HISTORY														
Patient's Name	Age of onset	Diag.	Cause	Other sensit.	Cause	Late or early	Results							
							1912		1913		1914		1915	
L. H. F.	21	L. H. F.	R. and G.	None.		L.				Pro.	+	Pro.	++	
	5	L. H. F.	R.	Urtic.		L.				Pro.	+			
		E. H. F.		Unk.										
L. H. F.	30	L. H. F.	R.	None.		L.				Phy.	++	Pro.	++	
		Vernal catarrh.	Neg. to Pollen.											
L. H. F.	17	L. H. F.	R. and G.	Urtic.	Fish.	L.				Pro.	-			
L. H. F.— ♂ Asth. OK.	20	Vernal catarrh.	Grass-p.											
E. H. F.	32	L. H. F.	R. and G.	Gastro-ent.	Crabs.	L.				Phy.	++	Pro.	++	
	15	E. H. F.	(-) Grass-p.	H. F. and Asth.	Horse.									
	21	L. H. F.	R. and G.			L.				Pro.	++	Pro.	++	
	15	L. H. F.	R. and G.	None.		L.				Phy.	+	Pro.	+	
OK.	23	L. H. F.	R. and G.	None.		L.				Phy.	+			
		E. H. F.	(-)											
OK.	22	E. H. F.	R. and G.	None.		L.				Phy.	++			
		♂ Asth.												
	L. H. F.													
OK.	37	L. H. F.	R. and G.	None.		L.				Phy.	+	Pro.	+	
	20	L. H. F.	R. and G.	None.		L.				Phy.	++			
		♂ Asth.												
	14	L. H. F.	R. and G.	Urtic.	Straw and Fish.	L.				Phy.	+			
L. H. F.	16	L. H. F.	R. and G.	H. F.	Horse.	L.				Phy.	+			
		♂ Asth.												
OK.	Baby	L.H.F.	R. and G.	None.		L.				Phy.	-			
OK.														
L. and L. H. F.	3	L. H. F.	R. and G.	Itch throat—	Fruit.	L.				Phy.	+			
		♂ Asth.												
OK.	15	Vernal catarrh.	Neg. to Pollen.	None.										
	22	L. H. F.	R. and G.	Urtic.	Fish.	L.				Phy.	-			
	38	L. H. F.	R. and G.			L.				Phy.	++			
	16	L. H. F.	R. and G.	None.		L.				Phy.	++			
		E. H. F.												

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Othe
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
253	M.	36	Neg.	Neg.
254	F.	48	Neg.	Neg.	Son of half sister.
255	F.	24	Neg.	Neg.	Bro.	27	Bro.	Urtic.-Straw.	29
256	M.	23	Father.	L. H. F.	Neg.	None.	Bro.	L. H. F.
257	F.	29	Father.	L. H. F.	Neg.	5 Sisters.	Sis.	L. H. F.
258	M.	26	Aunt.	L. H. F.	Neg.
259	M.	33	Neg.	Neg.
260	M.	27	Neg.	Neg.	2 Bros.	Bro.	L. H. F.
261	M.	42	Neg.	Neg.
262	F.	16
263	M.	54	Neg.	Neg.	4 child.
264	M.	55
265	F.	13
266	M.	28	Neg.	Mother.	Urtic—Straw.	Cousin.
267	M.	36	Neg.	Neg.
268	M.	26	Neg.	Mother.	Asth.
269	F.	38	Neg.	Neg.	4 Sibs.
270	F.	25	Neg.	Neg.	1 Bro.	1 Bro.	L. H. F.
271	M.	33
272	F.	35	Neg.	Neg.
273	M.	58	Son. Daugh #363. Daugh. Son. #460
274	M.	26	Neg.	Neg.
275	F.	42	Neg.	Neg.
276	F.	27	Neg.	Neg.
277	M.	2	Aunt.	E. and L. H. F.	Neg.
278	M.	51	Neg.	Neg.
279	F.	36	Neg.	Neg.	None.	1 Bro.	L. H. F.

PATIENT'S HISTORY															
Patient's Name	Diag.	Age of onset	Diag.	Cause	Other sensit.	Cause	Late or early	Results							
								1912		1913		1914		1915	
J. H. F. Asth.		16	L. H. F. Asth.	R. and G.	Urtic.-	Quinine.	L.					Phy.	++	Pro.	++
		41	L. H. F.	R.	None.		L.					Phy.	-		
		19	L. H. F. Asth.	R. and G.			L.					Phy.	++	Pro.	+++
		10	L. H. F. Asth.	R. and G.	Urtic.-	Unk.	L.					Phy.	++		
		18	L. H. F.	R. and G.	Urtic.	Straw.	L.					Phy.	++	Pro.	+
		7	L. H. F. Asth.	R. and G.	Urtic.- Gastro- ent.	Unk. Crabs.	L.					Phy.	+++		
		33	Vaso- motor Rhinitis.	Neg to pollen.											
		11	L. H. F.	R. and G.			L.					Phy.	+		
		34	L. H. F.				L.					Phy.	++		
				L. H. F. Asth.			L.					Phy.	+++	Pro.	++
K. th.		46	L. H. F. Asth.	R. and G.	Urtic.	Clams and Crabs.	L.					Phy.	++	Pro.	++
		38	L. H. F.	R.			L.					Phy.	+		
		10	Vernal catarrh.	R. and G.											
		13	L. H. F.	R. and G.											
		26	L. H. F. Asth.	R. and G.	None.		L.					Phy.	+	Pro.	++
		16	L. H. F.	R. and G.	None.		L.					Phy.	++		
		31	Urtic.												
		22	L. H. F. & Asth.	R.			L.					Phy.	-		
				Neg. to pollen.											
		34	L. H. F. E. H. F.	R. and G. Grass-p.			L.							Pro.	+
L. H. F. som. hinitis.		38	L. H. F.	R.	None.		L.							Pro.	++
		21	L. H. F.				L.					Phy.	++		
		35	L. H. F.	R.	None.		L.					Phy.	+	Pro.	-
		22	L. H. F.	R.	None.		L.					Phy.	+	Pro.	++
		31	Bron. Asth												
		30	L. H. F. Asth.	R. and G.	None.		L.					Phy.	-		
		31	L. H. F. Asth.	R.			L.					Phy.	++		

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Other
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
280	M.	20	Neg.	Neg.	2 Sibs.	22	None.		
281	M.	22	Neg.	Neg.		24			
282	F.	40	Neg.	Neg.	7 Sibs.		None.		
283	M.	33	Grand-M.	Asth.				1 Bro.	Erythema —Qui- nine. Food poi- soning.	39	
			Aunt.	E. H. F. and Horse Asth.					1 Sister.			
284	M.	45	Neg.	Neg.						
285	F.	40									
286	M.	51	Father.	Asth.	Neg.			1 Bro.	Asth.		
287	M.	52	Neg.	Neg.	4 Sibs.	49	1 Bro.	Asth.		Wife's Mother.
								50				
								57				
								60				Daugh. 13
288	F.	35	Uncle.	Asth.	Neg.	1 Bro.		None.			
289	F.	32				2 Sibs.		1 Bro.	L. H. F.		
290	F.	49									
291	M.	41		Grand-f.	Asth.	5 Sibs.		None.			
292	M.	5	Grand-f.	Asth.	Aunt.	L. H. F.	3 Sibs.		None.			2 Pat'l Cousins.
293	F.	53	Neg.	Neg.	2 Bro. (Twins).	45				
							1 Bro.	47				Mat. Cous in.
							1 Bro.	57				Nephew (Bro. # 3).
							1 Bro.	61	1 Bro.	Asth.— Horse.	49	Nephew.
							1 Bro.	63				
							1 Sister.	51	1 Sis.	H. F.— Ragw.	60	

PATIENT'S HISTORY														
relatives	Age of onset	Diag.	Cause	Other sensit.	Cause	Late or early	Results							
							1912		1913		1914		1915	
	14	L. H. F.	R. and G.			L.					Phy.	++	Pro.	++
	7	L. H. F. & Asth.	R. and G.	None.		L.					Phy.	++		
	39	Chronic Rhinitis.	Neg. to pollen.											
	30	L. H. F. & Asth.	R.	None.		L.							Pro.	++
	29	L. H. F. & Asth.	R. and G.			L.					Phy.	++		
	37	L. H. F. & Asth.	R.	Erythema	Straw	L.					Phy.	++	Phy.	++
	26	L. H. F.	R. and G.	None.		L.					Phy.	++	Pro.	++
Urtic- Straw.	37	L. H. F.	R and G.			L.					Phy.	++	Pro.	++
OK.		E. H. F.	Cured.											
	20	Bron. Asth.	Neg. to pollen.											
	16	L. H. F.	G-rod.	H. F.	{ Horse. Sachet. Orris. Horse.									
				H. F.										
	37	Bron. Asth.												
	4	Bron. Asth.		None.										
Asth.	22	Bron. Asth.	R. and R. T.	Asth.	{ Egg Al. and dog. H. F. and Asth.									
				H. F. and Asth.		Horse.								
Edema.	38	E. H. F.	Grass-p.	Urtic.	Unk.	E.							Pro.	+
E. H. F.														
E. H. F.														

CASE NO.	SEX	AGE	FAMILY HISTORY										
			Paternal		Maternal		Sibs normal		Sibs sensitized			Other	
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative	
294	M.	72											2 P a t. Cousins Grand- nephew.
295	F.	38	Neg.		Neg.		2 Sis.						
296	F.	35	Neg.		Neg.		1 Bro.		1 Sis.	L. H. F.			
297	F.	60											
298	M.	52	Neg.		Neg.								2 child. {26 24 1 child # 229. Wife's fam.
299	M.	18	Father # 295.	Horse Asth.	Neg.		2 Sibs.	26 24	None.				
300	M.	66	Neg.		Neg.		4 Bros.			None.			Wife's fam. Son # 345. 3 child.
301	M.	49	Neg.		Neg.								
302	M.	7	Neg.		Neg.		2 Sis.						
303	M.	35											
304	M.	54	Neg.		Neg.								
305	F.	9	Neg.		Neg.								
306	F.	23	Neg.		Uncle.	L. H. F.	1 Bro.		None.				Mat'l Cousin.
307	F.	17	Father. Grand-m.	Asth. E. H. F.	Mother. Uncle. Aunt.	Asth. E. H. F. L. H. F.	5 Sibs.		None.				
308	F.	20	Father. Uncle. Gt. uncle.	L. H. F. & Asth. L. H. F. L. H. F.	Neg.		3 Sibs.		None.				
309	M.	9											
310	M.	23	Uncle.	H. F.— Horse.	Mctner.	Asth.— Cats.	None.		None.				Pat. Cous- in.
312	M.	10	Uncle. Uncle.	H. F. Asth.	Neg.		None.		None.				
313	F.	44	Neg.		Neg.		8 Sibs.		None.				Husb. fam. 1 child (13)

PATIENT'S HISTORY													
relatives	Age of onset	Diag.	Cause	Other sensit.	Cause	Results							
						Late or early	1912		1913		1914		1915
Asth.	32	Bron. Asth.	Tests neg.	Erythema	{ Fish. Straw. }								
Asth.	6	Bron. Asth.	Tests neg.	Gastroent.	Fish.								
	34	H. F. and Urtic.	Wheat protein.	None.					Pro.	+++	Pro.	+++	
	50	Bron. Asth.	Grass-p.	None.		E.					Phy.	++	
OK. L. H. F.	44	Horse Asth. H. F.	Dog. neg.	Asth.	Horse.								
OK.	8	L. H. F.	Not tested.	None.									
Neg. L. H. F. OK.	10	Bron. Asth.	Grass-p.	None.									
	47	Bron. Asth.	Neg. to pollen.	None.									
	4	L. H. F.	R. and G.			L.					Pro.	+	
	8	L. H. F.		Urtic.	Honey.	L.					Pro.	+	
	5	L. H. F. E. H. F.	R. Neg. Grass-pollen.										
L. and L. F. F.	20	E. H. F. L. H. F.	Grass-p. R.			E. L.					Pro. Pro.	++ ++	
	9	E. H. F. E Asth.	Grass-p.	None.		E.					Pro.	+++	
	4	E. H. F. L. H. F. E Asth.	Grass-p. R. and G.	H. F.	Horse.	E. L.					Pro. Pro.	++ ++	
	13	Bron. Asth.											
F. F. Horse.	7	Bron. Asth. Hay fever.	Grass-p.	None.									
	5	E. H. F. L. H. F.	Grass-p. R. and G.	Ang. N. Ed.	Unk.	E.					Pro.	+++	
K.	9	E. H. F.	R and G.			L.					Pro.	-	
K.		L. H. F. E Asth.	Grass-p.			L.					Pro.	+	

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Other
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
314	M.	29	Grand-f.	Asth.	Aunt.	Urtic.	None.	..	None.		
315	F.	25	Father.	Urtic.	Grand-M.	L. H. F.	2 Sibs.	..	None.		Husb.
316	M.	33	Neg.	Mother.	Asth.	1 Bro.	..	1 Sister.	Asth.	..	No child.
317	M.	1	Neg.	Grand-f.	Asth.	None.	..	None.		Wife's fam.
			Father.	E. H. F.	Mother.	E. H. F. & Horse Asth.						Child (1).
					Grand-f.	H. F. and Asth.						
					Great-grand. f.	H. F.						
					Great uncle	H. F. & Asth.						
					Uncle.	H. F.						
					Aunt.	H. F.						
318	F.	40	Neg.	Neg.	2 Bros.	..	None.		Pat'l 2nd cousin.
319	F.	9	Grand-f.	L. H. F.	Grand-M.	E. H. F. and L. E. H. F.	None	..	1 Sis.	Hay fever.	..	6.....
			Grt.-grd.- M.	L. H. F.	Mother, 158.							
			Aunt.	E. H. F.	Aunt.	Asth—cats.						
320		Aunt.	Urtic.								
321	F.	32	Neg.	Neg.	1 Bro.	44	1 Sis.	Early Hay F.	37	Husb. fam. 2 Child.
322	F.	31	Neg.	Neg.	4 Sibs.	..	None.		
323	F.	60	Neg.	Mother.	L. H. F.	11 Sibs.	..	None.		
324	M.	14	Neg.	Mother.	Urtic— Straw.	2 Bros.	12 2	None.		
325	M.	16	Father.	Asth.	Neg.	1 Bro.	14	None.		Pat'l 2nd cousin.
326	M.	19	Neg.	Neg.	2 Sis.	17 12	None.		
327	M.	13	Father, *194.	E. H. F.	Neg.	None.	..	1 Sister.	E. H. F.	6	
			Uncle.	Asth.								
328	M.	45										
329	F.	34	Neg.	Neg.	7 Sibs.	..	1 Sis. *409.	L. H. F.		
330	F.	38	Neg.	Mother.	L. H. F.	4 Sibs.	..	None.		
331	F.	40	2 Uncles.	L. H. F. & Asth.	Neg.	8 Sibs.	..	None.		Pat'l 1st cousin.
332	F.	33	Neg.	Aunt.	L. H. F.	2 Sibs.	136 131	None		

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Other
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
333	F.	31	Uncle.	Urtic— Oatmeal.	Mother.	Urtic— Straw.	3 Sibs.	45 39 25	None.			
			Father	Urtic— Straw.								
334	F.	30	Neg.		Neg.		4 Bros.	35 33 25 23	None.			
335	M.	11	Neg.		Neg.		1 Bro.	5	1 Bro.	L. H. F.	7	
336	M.	10	Neg.		Neg.		4 Sibs.	20 17 16 5	1 Sis. (Twin).	Urtic.— fish.	17	
337	M.	24	Neg.		Mother. Aunt.	Asth. Asth.	None.		None.			
338	M.	47	Father.	L. H. F. & Asth.	Neg.		2 Bros.	54 & 57	None.			
339	M.	18	Neg.		Neg.		4 Sibs.		None.			
340	M.	45	Neg.		Neg.		2 Sibs.		None.			Nephew.
341	M.	45	Aunt.	Asth.	Mother.	Asth.	5 Sibs.	31 33 35 37 59	1 Sis.	Asth.	25	Wife's fam. 1 daugh. 2 Sons. 2 Sons.
342	M.	19	Aunt.	L. H. F. & Asth.	Mother.	L. H. F. & Asth.	None.		1 Sis.	Irreg. H. F. & Asth.	16	
343	M.	25	Neg.		Mother.	Urtic.= Honey.	5 Sibs.		None.			
344	F.	24	Uncle.	L. H. F.	Neg.							
345	M.	36	Father \$ 300.	Asth.	Neg.		3 Sibs.		None.			Niece.
346	M.	42	Aunt.	Fish.	Neg.		1 Sis.	26	None.			Wife's fam. 2 child.
347	F.	27	Father.	Urtic.	Neg.				1 Sis.	E. H. F.	36	
348	M.											
349	M.	32	Neg.		Neg.							Wife's fam. 2 child.

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Other
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
a350	F.	38	Neg.	Neg.	2 Bros.	..	1 Sis.	E. H. F.	..	4 Nieces and nephews (of sister & E. H. F.) Husb. fam Son (13)
b350	F.	30	Neg.	Mother.	E. and L. H. F.	3 Bros.	33 36 37	None.
351	F.	34	Unk.	Neg.	None.	..	None.
352	F.	39	Neg.	Uncle.	E. H. F.	2 Bros.	..	3 Bros.	Early H. F. E. and L. H. F.	..	Nephew.
353	M.	59	Neg.	Uncle and aunt. Neg.	4 Sibs.	..	1 Bro.	Asth.	..	Wife's fam 3 Child.
354	F.	21	Neg.	Mother 355.	E. and L. H. F. & Asth. L. H. F. cured.	2 Sibs.	18 & 20	1 Bro.	E. and L. H. F.	24
355	F.	43	Aunt. Grand-M.	L. H. F. Asth.	Neg.	2 Bros.	38 & 41	None.	Daugh. 354. Son. Wife's fam
356	M.	42	Neg.	Grand-M.	Asth.	5 Sibs.	1 child. 1 child. Wife's fam 2 Child.
357	M.	39	Neg.	Neg.	1 Sis.	44	1 Sis.	L. H. F.	42
358	F.	34	Neg.	Neg.	8 Sibs.	..	1 Bro. 1 Sis.	H. F. Gastritis— Straw.	56
359	F.	33	Neg.	Grand-f.	L. H. F.	2 Bros.	37 39	1 Sis.	E. & L. H. F.	35 30	Husb. fam
					Uncle.	L. H. F.			2 Bros.	E. and L. H. F.	34	2 Sods. 1 Son.
360	F.	46	Neg.	Neg.	4 Sibs.	35 40 42 44	None.	1 Son. Husb.
												Husb. Sis. 1 Daught. 1 Son.
361	F.	31	Neg.	Neg.	3 Sibs.	..	None.	Husb. 2 Child. 1 Child.

PATIENT'S HISTORY														
Patient's Name	Age of onset	Diag.	Cause	Other sensit.	Cause	Late or early	Results							
							1912		1913		1914		1915	
H. F.	38	Rhinitis.	
OK. Bron. Asth.	5	E. H. F. L. H. F.	Neg. to pollen.	Erythema Gastritis.	Straw. Onions	
.....	2	L. H. F.	R. Neg. to Grass-p.	Gastritis.	Fish.	L.	Pro.	+	
.....	9	E. H. F. E. H. F.	Grass-p.	E.	Pro.	++	
Free. OK.	24	E. H. F.	Grass-p.	Urtic.	Straw.	E.	Pro.	+++	
.....	12	E. and L. H. F. ♂ Asth.	Grass-p. R. and G.	None.	E. L.	Pro.	+	
.....	22	E. and L. H. F. ♂ Asth.	Grass-p. Ragw.	E. L.	Pro.	++	
OK.	12	E. and L. H. F. ♂ Asth.	Grass-p. and R.	Urtic.	Unk.	E. L.	Pro.	++	
OK.	19	L. H. F. E. H. F. ♂ Asth.	Grass-p. and R.	None.	E. L.	Pro.	+++	
.....	17	L. H. F. E. H. F.	R. and G. Daisy.	None.	L.	Pro.	+	
OK.	17	L. H. F.	R. and	
OK. and L. H. F. H. F. F. and Horse Asth.	11	L. H. F. E. H. F.	R. and G. Grass-p.	None.	L.	Pro.	+	
OK. ng. n. ed. and H. F. eg. K. H. F.	8	E. H. F.	Grass-p. R.	Urtic.	Unk.	E.	Pro.	+	

PATIENT'S HISTORY													
Diag.	Age of onset	Diag.	Cause	Other sensit.	Cause	Results							
						late or early	1912		1913		1914		1915
	56	Bron. Asth.	Neg to pollen.										
	17	E. H. F.	Grass-p.	None.		E.						Pro.	++
	2	L. H. F.	Grass-p. R.			L.						Pro.	++
	5	E. H. F.	Grass. R.	None.		E.						Pro.	++
		L. H. F.	R.			L.						Pro.	+
F. & Asth.	12	L. H. F. with Asth.	R.	None.		L.						Pro.	+
F. & Asth.	12	Dermatitis.	Poison Ivy.										
F.													
H. F.	40	E. H. F.	Grass.	Urtic.	Fish.	E.						Pro.	+++
H. F.		ẽ Asth.				L.						Pro.	+++
t. Fish. F.		L. H. F.	R.										
H. F. tic. tic.		H. F.	Unck.	H. F. and Asth.	Horse.								
	20	E. H. F.	Grass.			E.						Phyl. Pro.	+++
		L. H. F. & Asth.	R. and G.			L.							-
	17	E. H. F.	Sp.			E.						Phy.	+
h.	17	E. H. F.	Sp.			E.						Phy.	+
A. S. F. ob.	24	L. H. F. & Asth.	R.			L.						Pro.	++
	23	E. H. F.	G. P.										
		L. H. F. & Asth.	R. and G.			L.						Pro.	+++
	22	E. H. F.	G. P.			E.						Phy.	+++
		L. H. F.	R.			L.						Pro.	+++
	26	L. H. F.	R.			L.						Pro.	+++

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Other
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
378	F.	27										Husb. B. Child—7 mos.
379	M.	27	Neg.		Neg.		B.	30	None.			Wife. 1 child 10 mos.
380	M.	26	F.	L. H. F.	M.	L. H. F.			B.	Urtic.—Unkn.	28	
									B.	Urtic.—Unkn.	22	
381	F.	60										
382	M.	40	Neg.		Neg.		S.	43	None.			Wife's Fam. 1 child.
							S.	38				
383	M.	27	Neg.		Neg.		B.	30				
384	F.	60	G. M.	H. F.	Neg.		B.	28				
385	M.	8	F. #112.	E. H. F.	M. #31.	L. H. F.	S.	10	None.			
386	M.	42	Neg.		Neg.		7 B. and S.		None.			W.'s Fam. No child.
387	M.	31	Neg.		Neg.		4 B. and S.		None.			
388	F.	33	Neg.		Neg.		10 B. and S.		None.			
389	M.	45										
390	M.	35	F. #391.	E. H. F.	M.	Urtic—Straw.	S.	48	None.			
							S.	46				
							B.	44				
391	M.	77	F.	Asth.	Neg.							W. Urtic—Child see
392	F.	44	Neg.		M.	Asth.	4 B. and S.		None.			
393	M.	21	A. U.	Asth. E. H. F.	Neg.		B. (twin)	27	S. (twin)	E. H. F.	27	
							B.	25				
							S.	19				
							B.	15				
394	M.	28	Neg.		M.	E. H. F.	S.	29	S.	L. H. F.	25	
							B.	23				
395	M.	22	Neg.		Neg.		S.	23	None.			
							B.	18				
							B.	16				
396	F.	38										
397	M.											
398	M.	21	Neg.		M.	Urtic—F.	B.	32	S.	Urtic—Straw.	24	
399	F.	13	F. U.	Urtic-Unk. L. H. F.	2A.	G. Ent—F.	None		S # 400 B. # 187.	L. H. F. E. and L. H. F.	14 6	

PATIENT'S HISTORY														
Patient	Age of onset	Diag.	Cause	Other sensit.	Cause	Results								
						Later or earlier	1912		1913		1914		1915	
L. H. F. OK.	24	E. H. F.				E.							Phy.	++
OK. OK.	16	L. H. F. & Asth.	R. and G.											
	17	L. H. F.	R.	Urtic.	Unk.									
		L. H. F.	R. and G.			L.							Pro.	+++
OK.	30	L. H. F.	R.			L.							Pro.	++
OK.	22	L. H. F.	R. and G.			L.							Pro.	++
	20	E. H. F.	G. P.			E.							Phy.	+++
		L. H. F.	R.			L.							Pro.	+++
	5	E. H. F.	G. P.											
		L. H. F. & Asth.												
OK.	28	L. H. F.	R.	None.		L.							Pro.	++
	7	E. H. F.	G. P.			E.							Phy.	++
	23	E. H. F.	G. P.	None.		E.							Phy.	+++
		E. H. F. & Asth.	G. P.			E.							Phy.	++
	8	L. H. F.	R.											
Draw. 390.	60	E. H. F.	G. P.			E.							Phyl.	++
	14	E. H. F.	G. P.											
	4	E. H. F.	G. P.											
		L. H. F. & Asth.	R.			L.							Pro.	++
	15	E. H. F.	G. P.	None.		E.							Phy.	++
	17	E. H. F.	G. P.	None.										
		L. H. F.	R.			L.							Pro.	++
	34	E. H. F.	G. P.			E.							Phy.	++
		L. H. F.	R.											
		Neg. to pollen.												
	19	B. Asth.	Neg. to pollen.	None.										
	9	L. H. F.	R. and G.			L.							Pro.	++

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Othe
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
400	F.	14	F. U.	Urtic-Unk. L. H. F.	2A.	G. Ent. F.	None.	..	S #399. B. # 187.	L. H. F. E. and L. H. F.	13 6
401	M.	14	F.	L. H. F. Urtic.	M. g'f. A. U.	Asth-Unk. Urtic Unk. Asth— Horse. L. H. F. L. H. F.	B.	33	S.	Asth-Cat.	28
402	M.	22	Neg.	Neg.	5 B. and S.	..	None.
403	M.	47	F.	Asth.— Unk.	Neg.	S.	46	None.	W.'s fam Son. 20.
404	M.	31	Neg.	Neg.	S.	30	None.	W. S.—4 yrs.
							S.	28				
							B.	24				
							S.	19				
							B.	17				
405	M.	31	Neg.	Neg.	B.	35	None.	W.—
406	F.	41	Neg.	Neg.	S.	45	B.	Asth.	48	Husb. an fam. D.—8 yrs
							S.	34				
407	M.	11	F.	E. and L. H. F.	Neg.	S.	20	B. # 408.	E. and L. H. F.	33
							B.	14				
408	M.	33	F.	E. and L. H. F.	Neg.	S.	20	B. # 407.	L. H. F.	11	W. 3 Sons.
							B.	14				
409	F.	36	Neg.	Neg.	7 B and S.	..	S. # 329.	E. H. F.	34	H. Urtic. No child.
410	M.	43	Neg.	Neg.	None.	..	B.	Urtic. Straw.	27	W. and far Son.
411	F.	40	Neg.	Neg.	B.	30	S.	Asth.	45
									S.	E. and L. H. F.	27
412	F.	26	Neg.	M.	L. H. F. and Asth.	None.	..	None.	H.
												2 Child.
413	M.	45	Neg.	Neg.	4 B. and S.	..	B.	H. F.
414	M.	51	g't-g'm. F.	Asth. Asth.	Neg.	S.	34	None.	W.'s fam. S.—28
415	F.	44	Neg.	Neg.	D.—18. H.'s fam. S. 20. S. 19. d. 17. S. 14.

PATIENT'S HISTORY														
relatives	Age of onset	Diag.	Cause	Other sensit.	Cause	Late or early	Results							
							1912		1913		1914		1915	
	10	L. H. F.	R.	None.		L.						Pro.	++	
	9	E. H. F. L. H. F.	G. P. R.	H. F.	Chicken.	E. L.						Phy. Pro.	++ +++	
	18	B. Asth.	Unk.											
O.K.	27	L. H. F.	R.	G. Ent.	Fish.	L.						Pro.	++	
O.K.	15	L. H. F. ♂ Asth.	R.	G. Ent.	Peach.	L.						Pro.	++	
	10	E. H. F.	G. P.	Urtic.	Unk.	E.						Phy.	+++	
	23	L. H. F. ♂ Asth.	R.	None.		L.						Pro.	+++	
urtic.	8	L. H. F.	R.	None.		L.						Pro.	+++	
	7	E. H. F.	G. P.			E.						Phy.	++	
K.		L. H. F. ♂ Asth.	R.			L.						Pro.	++	
raw.	31	L. H. F.	R. and G.	H. F.	Violet Sachet.	L.						Pro.	++	
K.	28	L. H. F.	R.	None.		L.						Pro.	++	
K.	39	E. H. F. L. H. F.	G. P. R.			E. L.						Phy. Pro.	+++ +++	
K.	16	L. H. F. and Asth.	R.	H. F.	Cat.	L.						Pro.	+	
K.	16	L. H. F. and Asth.	R.	Urtic.	Unk.	L.						Pro.	++	
K. H. F. and br. Asth.	33	E. H. F. L. H. F. and Asth.	G. P. R. and G.	G. Ent.	Clam.	L.						Pro.	+++	
K. K. H. F. H. F. K. K.	38	L. H. F.	R.	None.		L.						Pro.	++	

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Othe
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
416	M.	31	U.	Urtic— Straw.	Neg.	S. B.	30 28	S. S.	L. H. F. Urtic.— Unk.	32 26	W. No child.
417	F.	18	Neg.	A.	L. H. F.	B.	21	B.	E. H. F.	23
418	M.	40
419	M.	13	Neg.	A.	E. H. F.	S.	10	None.
420	M.	30	Neg.	A. Neg.	E. H. F.	S.	32	None.
421	M.	47	Neg.	Neg.	3 B. 4 S.	..	None.	W.'sfam. D. 16. 5 S. and 1
422	F.	38	Neg.	Neg.
423	M.	29	Neg.	Neg.	8 B. and S.	..	B.	Asth.	33
424	F.	49	Neg.	Neg.
425	F.	46	G'm.	Asth.	Neg.	8 B and S.	..	None.	H. 2 child.
426	M.	31	Neg.	Neg.	S. S.	20 17	None.	W.'s A. No child W.'s fam 8 Child.
427	M.	63	Neg.	Neg.
428	M.	47	F.	Asth.	Neg.	B.	49	None.	W.
429	F.	43	Neg.	Neg.	S. S. S.	41 39 37	None.	H. 1 Child.
430	M.	62	F.	H. F.— Corn.	Neg.	4 B. and S.	..	None.	W.'s Fam
431	M.	58	No child W.'s Far 4 child. 2 child.
432	F.	27	Neg.	Neg.	2 Sis.	..	None.	H.'s fam 2 child.
433	M.	37	Neg.	Neg.	L.	S. S. Asth. (cured).	45 41	S. B.	H. F. H. F. c.	49 47	W.'s fam No child
434	M.	35	Neg.	Neg.	B. S.	32 18	None.
435	M.	35	Neg.	U. g'f.	Asth. Asth.	3 B. and S.	..	None.	D. #58

		PATIENT'S HISTORY												
relatives		Age of onset	Diag.	Cause	Other sensit.	Cause	Results							
Diag.	Late or early						1912		1913		1914		1915	
OK.	23	L. H. F. and Asth.	R.	Asth.	Horse.	L.							Pro.	+++
	17	Vaso U. Rhinitis.	Neg. to Pollen.	None.										
	36	L. H. F.	R.											
	12	E. H. F.	G. P.	Urtic.	Unk.	E.							Phyl.	+++
	25	E. H. F. L. H. F. ♂ asth.	G. P. R.			L.							Pro.	+
OK. L. H. F. OK.	29	L. H. F. ♂ Asth.	R. and S.			L.							Pro.	++
	24	E. H. F. L. H. F. ♂ Asth.	R. and G.			L.							Pro.	++
	9	L. H. F. ♂ Asth.	R. G. P.	Eryth.	Fish.	L.							Pro.	+++
	41	Pulm. tb. B. Asth.		None.										
OK.	14	E. H. F.	G. P.	H. F. and Asth.	Pigeon.									
OK.		L. H. F. Asth.	R. and G.	H. F. and Asth. Edema.	Canary. Unk.	L.							Pro.	++
Asth.	7	L. H. F.	R.			L.							Pro.	+
Neg. OK.	48	L. H. F. ♂ Asth.	R.	None.		L.							Pro.	++
OK.	15	L. H. F.	R. and G.	Urtic. Eryth.	Unk. Fish.	L.							Pro.	++
OK. OK.	10	L. H. F.	R.	None.		L.							Pro.	++
Neg.	32	L. H. F. ♂ Asth.	R.	None.		L.							Pro.	++
Neg. OK. L. H. F. OK. OK.	52	L. H. F.	R.	None.		L.							Pro.	-
	12	L. H. F.	R. and G.	Urtic.	Fish.	L.							Pro.	++
	30	L. H. F.	R.	Urtic.	Unk.	L.							Pro.	+
	23	L. H. F.	R.	None.		L.							Pro.	++
L. H. F.	12	E. H. F. L. H. F.	G. P. R.	H. F. and Asth.	Horse.	E. L.							Phyl. Pro.	++ ++

		PATIENT'S HISTORY												
relatives	Age of onset	Diag.	Cause	Other sensit.	Cause	Results								
						Late or early	1912		1913		1914		1915	
	5	E. H. F. L. H. F.	G. P. R.			E. L.							Phyl. Pro.	+++ ++
	8	E. H. F. L. H. F.	G. P. R.	Urtic. G. Ent.	Egg. Egg.	E. L.							Phyl. Pro.	++ ++
OK. E. and L. H.F.	23	E. H. F. ♂ Asth.	G. P.	Urtic.	Unk.	E.							Phyl.	+++
OK.		L. H. F.	R.											
	30	L. H. F. ♂ Asth.	R. and G.	None.										
	6	L. H. F.	R.			L.							Pro.	+++
OK. OK.	32	E. H. F. L. H. F.	Neg. to G. P.	None.		L.							Pro.	++
	5	E. H. F. L. H. F.	Grass-p. R.											
OK.	39	L. H. F. ♂ Asth.	R. & G.			L.							Pro.	++
	36	L. H. F.	Grass-p. R.			L.							Pro.	+++
OK.	4	L. H. F.	R.			L.							Pro.	+
OK.	4	E. H. F.	Grass-p.											
OK.	4	L. H. F.	R.			L.							Pro.	++
OK.	2	L. H. F. ♂ Asth.	R. and G.	None.		L.							Pro.	+
OK. OK.	25	L. H. F.	R. and G.	None.		L.							Pro.	+++
Asth.	38	L. H. F.	R. and G.			L.							Pro.	+
OK. OK.	29	L. H. F. E. H. F.	R. and Grass-p.	H. F.	Cat and parrot.	L.							Pro.	++
Urtic. OK.	48	L. H. F. E. H. F.	R. and Grass-p.			L.							Pro.	+++
	9	E. H. F.				E.							Phy.	++

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Othe
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
453	M.	47	Neg.	Neg.	6 Sibs.	..	None.	Wife. 2 Child.
454	M.	57	Neg.	Neg.	9 Sibs.	..	None.	Husb. fam 2 Child.
455	M.	38	Aunt	Asth.	3 Sibs.	..	1 Bro.	H. F. (cured).	..	Wife's fam Son #3.
456	M.	46	Neg.	Neg.	Daugh. 1 child.
457	F.	29	A.	E. H. F.	Neg.	B. S. S. S.	50 44 42 36	B. #129.	H. F.	46
458	F.	36	Neg.	M. gm. U. A.	Urtic—fish L. H. F. L. H. F. Urtic—fish	None.	..	None.	H. No Child
459	M.
460	M.	15	F. #273.	L. H. F.	Neg.	B.	22	S. #363. S.	E. H. F. Urtic— straw.	21 19
461	M.	25	U.	Asth.— horse.	M.	L. H. F.	B. S. S.	27 23 19	None.
462	F.	54	Neg.	Neg.	6 B. and S.	..	None.	H. 1 child 2-
463	M.	47	Neg.	Neg.	None.	..	S.	H. F. & Asth.	49
464	M.	45	F. 2 U.	Asth. Asth.	Neg.	2 B.	Asth.
465	M.	55	Neg.	Neg.	8 B. and S.	W's fam. 2 child.
466	M.	26
467	M.	45	Neg.	Neg.	S.	50	B.	L. H. F.	48
468	M.	46	U.	Asth.	g. f.	Asth.	None.	..	S.	Urtic— Straw.	45

PATIENT'S HISTORY														
relatives	Age of onset	Diag.	Cause	Other sensit.	Cause	Late or early	Results							
							1912		1913		1914		1915	
OK.	37	L. H. F.	R. and G.	Urtic.	Shell	L.						Pro.	++	
OK.		ē. Asth.	Gross-p.		fish.									
OK.	31	L. H. F.	R.	None.		L.						Pro.	++	
OK.														
OK.	6	Bron.-		Gastro-ent.	Egg-									
H. F. fr.		Asth.			White.									
Ragw.														
and Gas-														
tro-ent.														
fr. Egg-														
White.														
H. F. fr.														
Grass-p.														
OK.	12	B. Asth.		H. F. and	Horse.									
		E. H. F.	G. P.	Asth.										
	6	L. H. F.	R.	Urtic.	Unk.	L.						Pro.	+++	
		and	G. P.											
		Asth.												
OK.	15	B. Asth.												
		E. H. F.	G. P.	Urtic.	Straw.									
		L. H. F.	R. and G.			L.						Pro.	+	
		L. H. F.												
		and												
		Asth.												
		V. motor	Neg to											
		Rhinitis.	Pollen.											
	7	E. H. F.	G. P.	H. F.	Horse.									
		L. H. F.	R. and G.			L.						Pro.	++	
		and												
OK.	34	L. H. F.	R. and G.	None.		L.						Pro.	++	
OK.		and												
		Asth.												
	40	B. Asth.	Neg to											
			Pollen.											
	27	B. Asth.	Neg to	None.										
			Pollen.											
OK.	40	L. H. F.	R. and G.	Urtic.	Unk.	L.						Pro.	+++	
OK.														
	3	E. H. F.												
		L. H. F.	R. and G.			L.						Pro.	++	
		and												
		Asth.												
	33	L. H. F.	R.	Non		L.						Pro.	+++	
	31	L. H. F.	R.	None.		L.						Pro.	++	
		and												
		Asth.												

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CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Other
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
503	M.	35	Neg.	Neg.	None.	..	None.	Wife's fa 1 child.
504	M.	55	Neg.	Neg.	8 Sibs.	..	None.	Wife's fa 4 child.
505	F.	26	Father.	E. and L. H. F. & Asth.	Neg.	None.	..	None.	
506	M.	60	Neg.	Neg.	Yes?	..	None.	4 child.
507	F.	40	2. 2 Sis.	..	1 Bro.	H. F.	..	Husb. fa Son 508. 4 child.
508	M.	16	Neg.	Mother s 507.	L. H. F.	4 Sibs.	..	None.	
509	M.	20	Neg.	Neg.	3 Bros.	..	None.	
510	M.	42	Neg.	Neg.	2 Sibs.	36 41	None.	
511	F.	30	Neg.	Mother.	Urtic— Straw.	1 Bro.	32	None.	
512	M.	42	Neg.	Mother. Grand-m.	E. H. F. E. H. F.	1 Sis.	39	1 Sis.	L. H. F. & Asth.	35	
513	M.	44	Neg.	Neg.	6 Sibs.	..	1 Bro.	H. F.	..	Wife. 1 child. 1 child.
514	M.	38	Neg.	Aunt.	L. H. F.	?	..	1 Bro.	Horse Asth.	..	Mat'l niece.
515	F.	37	Neg.	Neg.	2 Sibs.	34 36	None.	Pat'l nephew Husb. s ter. 2 child.
516	F.	40	Neg.	None.	..	None.	Mat'l aunts. Wife. No child
517	M.	35	Father.	L. H. F.	Neg.	None.	..	Sister. Bro.	L. H. F. L. H. F.	32 27	Wife's fa 4 child.
518	M.	8	Neg.	Neg.	
519	M.	34	Neg.	Grand-f. Aunt.	Asth. L. H. F. & Asth.	5 Sibs.	..	None.	Wife. 1 child.
520	M.	34	

PATIENT'S HISTORY													
Diag.	Age of onset	Diag.	Cause	Other sensit.	Cause	Results							
						Later or early	1912		1913		1914		1915
	7	L. and E. H. F.	Neg to R. and G. and Grass-p.	None.								(Cured?)	
	43	E. H. F. ♂ Asth.	R.	None.		L.						Phy.	+
	7	E. H. F. L. H. F.	R. (-)	None.		L.						Phy.	++
		E. H. F. (-).	R. and G. Grass-pollen.			L.						Phy.	+++
	35	L. H. F. E. H. F. (-).	R. Grass pollen.			L.						Phy.	+++
	22	L. H. F.	R. and Grass-p.	Erythema	Fish.	L.						Phy.	+++
	18												
	8	L. H. F. E. H. F. (-).	Grass-p. R. and G.	None.		L.						Phy.	
	7	L. H. F. ♂ Asth.	R. and G.	None.		L.						Phy.	+++
	5	L. H. F. ♂ Asth.	R. and G.			L.						Phy.	+++
	23	L. H. F. E. H. F. (-).	R.	Ang. N. Ed. Urtic.	Straw. Sea-food	L.						Phy.	+++
	10	L. H. F. ♂ Asth.	R.	Erythema.	Straw.	L.						Phy.	+++
	31	L. H. F. ♂ Asth.		None.									
	27	L. H. F. ♂ Asth.	R.	None.		L.						Phy.	++
	32	L. H. F.	R.	None.		L.						Phy.	++
	23	L. H. F.											
		♂ Asth.	R.	None.		L.						Phy.	+
	20	L. H. F.	R. and G.			L.						Phy.	+
	3	Poison Ivy.											
	28	L. H. F.	R. and G.	Urtic.	Peaches.	L.						Phy.	(-)
	7	L. H. F. ♂ Asth.	R. and G.			L.						Phy.	++

PATIENT'S HISTORY														
relatives	Age of onset	Diag.	Cause	Other sensit.	Cause	Late or early	Results							
Diag.							1912		1913		1914		1915	
OK. OK.	35	L. H. F.	R.	None.		L.							Phy.	+++
		L. H. F.												
	33	L. H. F.	R.	None.										
OK. Urtic. OK.	22	L. H. F. & Asth. E. H. F. (-)	R.	Urtic. H. F.	Unk. Horse.	L.							Phy.	++
OK. OK.	28	L. H. F.	R.	None.										
L. H. F.	26	Vaso-mo- tor Rhi- nitis.	Neg to pollen. Pos. to Orris.	Urtic.	Clams, Straw.									
OK.	16	L. H. F.	R.	None.		L.							Phy.	++
	13	L. H. F.	R.	H. F. and Asth.	Horse.	L.							Phy.	++
	10	L. H. F. & Asth.	R. and G.			L.							Phy.	++
	3	L. H. F. & Asth.												
	3	Bron- Asth.	Grass-p. R. and dog.			L.							Phy.	++
	39	L. H. F.	R. and G.			L.							Phy.	+
	24	Vaso- motor Rhinitis.	Neg to Pollen.											
OK. ?	15	L. H. F. & Asth.	R. and G.	None.		L.							Phy.	+++
	24	L. H. F.	R.			L.							Phy.	++
	33	L. H. F. E. H. F. (-)	R. and G. Grass-p.			L.							Phy.	+
	15	L. H. F.	R. and G.	Gastro- ent. and urtic.	Clams and Straw.	L.							Phy.	++
	23	L. H. F.	R.	None.										
	18	L. H. F.	R. and G.			L.							Phy.	+

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Other
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
540	M.	9	Father.	E. H. F.	Mother.	Asth. Horse. E. H. F. Gastro- ent. and Urtic. Straw and Clams. Asthma. Asthma.	1 Bro.	5	1 Sister.	Urtic— Unk.	12	
541	F.	30										
542	M.	28										
543	M.	15	Neg.		Mother.	Urtic— Straw.	2 Sis.	8 19	1 Bro. # 537. 1 Bro. # 5.	L. H. F. H. F. and Asth.	17 12	
544	M.	46	Neg.		Neg.		3 Bros.	26 36 & 44	None.			Wife can't eat lob- ster. 3 child. Mat'l cousin.
545	F.	40	G'm. F.	Asth. Asth.	G. F.	L. H. F.	B.	37	None.			
546	F.	27	F.	Uc. G. Ent. clams.	Neg.		None.		B.	E. H. F.	30	
547	M.	42										
548	M.	43	F. G. F.	L. H. F. L. H. F. Asth.	Neg.		3 B. and S.	27 47 54	S.	E. H. F.	58	W's urtic W's U. 1 child.
549	F.	31										
550	M.	22										
551	M.	12	F.	Urtic sea food.	M.	E. H. F. Urtic Straw.	None.		None.			
552	M.	47	Neg.		M.	Asth.	2 B.		None. •			Wife. 5 child.
553	M.	24	Neg.		M.	Urtic Straw.	B.	28	None.			W. No child
554	M.	23	Unkn.		Unkn.		None.		None.			None.

PATIENT'S HISTORY													
Patient's name	Age of onset	Diag.	Cause	Other sensit.	Cause	Results							
						Late or early	1912		1913		1914		1915
	8	Asth.?	Neg. to pollen.	Urtic.	Fish.								
	29	L. H. F.	R.										
	9	L. H. F.	R.			L.						Pro.	++
	11	3 Asth. L. H. F.	R.			L.						Phyl.	++
K.	31	L. H. F. and Asth.	R.	G. Ent. G. Ent. Urtic. H. F.	Clams. Fish. Onions. Tannipa.	L.						Phyl.	++
L. F.	15	L. H. F. and Asth.	R.										
	23	E. H. F. L. H. F.	G. P. R.	Urtic. G. Ent.	Straw. Crab.	L.						Phyl.	+
Straw. F. K.	37 2	L. H. F. E. H. F. L. H. F. and Asth.	R. G. P. R.	Urtic.	S-fish.								
	29	E. H. F. L. H. F. and Asth.	G. P. R. and G.			L.						Phyl.	++
	19	L. H. F.	R. and G.			L.						Phyl.	+++
	5	L. H. F.	R.			L.						Phyl.	++
K.	27	B. Asth.		None.		L.						Phyl.	-
K.	38	L. H. F. and Asth.	R. and G.										
		E. H. F.	G. P.										
K.		E. H. F.	G. P.	None.		L.						Phyl.	+
		L. H. F.	R. and G.										
	22	L. H. F.	R. and G. G. P.	Urtic.	Straw.	L.						Phyl.	+++

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Other
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
555	F.	26	G. M.	Asth.	Neg.		2 B. and S.		None.			
556	M.	53	Neg.		Neg.		None.		B.	Asth.	58	
557	M.	40										
558	F.	35	Neg.		Neg.		5 B. and S.		B.	H. F. (cured).		Husb. 2 child.
559	M.	31	Neg.		Neg.		2 B. and S.		None.			W. No child.
560	M.	22	Neg.		Neg.		S.		None.			
561	F.	26							B.	L. H. F.		
562	M.											
563	M.											
564	M.	39										
565	F.	32	Neg.		Neg.		None.		None.			
566	M.	38	Neg.		U.	L. H. F.	None		None.			mat'l cousin. W. 1 child.
567	M.	18	Neg.		Neg.		7 B. and S.		S.	H. F. and Asth.		
568	M.	13	Neg.		Neg.		4 B. and S.		None.			
569	F.	44										
570	M.	18	A.	L. H. F.	G. M.	E. H. F. and Asth.	2 Sis.		None.			
571	F.	55	U.	Asth.	A. M.	Urtic. Asth.	B.	49	B.	H. F. and Asth.	40	Hus.
			F.	Urtic.	G. M.	Urtic. Asth.			B. S.	Urtic. Urtic.	45 52	3 child.
572	M.	12	F.	G. Ent. Shell fish.	M.	L. H. F. and Asth. Urtic- S-fish. Asth.	2 B. and S.	21 22	None.			
573	M.	22	Neg.		A. Neg.		S.		None.			

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CASE NO.	SEX	AGE	FAMILY HISTORY										
			Paternal		Maternal		Sibs normal		Sibs sensitized			Other	
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative	
574	F.	29	F.	L. H. F.	Neg.								
575	M.	30	Neg.		Neg.		6 B. and S.		None.				
576													
577	F.	38	3 U.	L. H. F.	Neg.								Pat'l Cousin. mat'l cousin. Husb. 3 child. 4 child. #579 and #580.
578	F.	46										2	
579	F.	16	Neg.		M. #578.	L. H. F. and Asth.	2 B. and S.	20 28	S. #580.	E. H. F.	26		
580	F.	26	Neg.		M. #578.	L. H. F. and Asth.	2 B. and S.	20 28	S. #579.	L. H. F.	16		
581	F.	33	F.	Urtic— Straw. Asth.	Neg.		None.		S.	Horse— Dog— Cat.	35	Pat'l Cousin. #215.	
582	F.	9	U. F. #435. G. M. Gt. G. F.	E. and L. H. F. Asth. Asth.	Neg.								
583	M.	29											
584	M.	40											
585	M.	37											
586	M.	28	Neg.		Neg.		2 B. and S.		None.			W. 2 child.	
587	F.	17	F.	Urtic buck- wheat.	Neg.								
588	M.	62										3 child.	
589	M.	39	Neg.		Neg.								
590	F.	32	Neg.		N.	Asth.			B.	E. and L. H. F.		Baby urtic an asth.	
591	M.	51							B.	E. H. F.			
592	F.	34	Neg.		Neg.							No child.	

PATIENT'S HISTORY														
Patient's Name	Age of onset	Diag.	Cause	Other sensit.	Cause	Laboratory early	Results							
							1912		1913		1914		1915	
L. F.	13	L. H. F.	R.			L.						Phyl.	++	
L. F.	25	L. H. F. and Asth.	R. and G.											
L. F.	30	L. H. F.	R.	None.		L.						Phyl.	++	
L. F.														
L. F.	30	L. H. F. and Asth.	R. and G.			L.						Phyl.	++	
L. F.														
L. F.	14	L. H. F.	R.			L.						Phyl.	++	
L. F.														
L. F.	21	E. H. F.		Urtic.	Straw.								++	
L. F.	11	B. Asth. E. H. F. L. H. F.	G. P. R.	H. F. and Asth. Asth.	Dog. Cat. Horse.	L.						Phyl.	-	
L. F.	9	L. H. F.												
L. F.	17	L. H. F.	R.			L.						Phyl.	++	
L. F.	27	L. H. F.	R.			L.						Phyl.	++	
L. F.	34	L. H. F.	R.											
L. F.	7	L. H. F. E. H. F. (cured).	R. G. P.	Urtic.	Unk.	L.						Phyl.	++	
L. F.	15		R. and G. G. P.											
L. F.	47	L. H. F. and Asth.	R.											
L. F.	38	L. H. F.	R. and G.											
L. F.	19	L. H. F.	R.	H F.	Horse									
L. F.	27	E. H. F.	G. P.											
L. F.	26	B. Asth. L. H. F.	G. P. R.											
L. F.	16	L. H. F. and Asth. E. H. F. and Asth.	R. and G. O. P.	H. F. and Asth.	Celery.									

[illegible]

[illegible]

CASE NO.	SEX	AGE	FAMILY HISTORY									
			Paternal		Maternal		Sibs normal		Sibs sensitized			Oth
			Relative	Diag.	Relative	Diag.	Relative	Age	Relative	Diag.	Age	Relative
613	F.	3	Neg.	M.	L. H. F. and asth.	Pat'l U. Mat'l U 1 child.
					U. GT. U. G. M.	Asth. Asth. E. H. F.						1 Boy. 1 Boy.
614	M.	21	F.	L. H. F.	Neg.	3 B.	6 B. 13 19	L. H. F.	16
615	M.	22	Neg.	Neg.	2 B.	..	None.
616	F.	39	Neg.	Neg.	2 B. and S.	..	None.
617	F.	48	Neg.	Neg.	2 B. and S.	..	None.	Husb. far 2 Boys.
618	M.	47	Neg.	M.	Asth.	2 B.	42 B. 49	H. F.	15	Wife and fam. No. child
					GT. U. A.	E. and L. H. F. Asth. L. H. F.						
619	F.	31	Neg.	Neg.	4 B. and S.	28 S. 34 40 43	H. F.	6
620	M.	34	Neg.	Neg.	B.	40	None.	W. and fam. 3 child.
621	F.	36	Neg.	Neg.	B. None.	46 ..	None.	Husb. 2 child.
622	F.	13	Neg.	A.	Asth.	B (twin).	18	B. (twin).	H. F. and Asth (Horse).	18
623	F.	44	F.	B. Asth.	G. F.	Asth.	B. B.	38 33	None.	No child.



A STUDY OF THE GROUPING OF MENINGOCOCCUS STRAINS

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In the spring of 1911, the members of the meningitis department started to investigate the possible grouping of strains of the meningococcus, being led thereto by Torrey's (1) success in demonstrating the division of strains of gonococcus into groups. The desirability of some definite knowledge on this point is obvious. Not only is it of academic interest, but in the preparation of anti-meningitis serum, it is of the utmost importance that, if the strains differ in their serological reactions and may be classified thus, as many groups as possible should be represented. Also in view of the large number of strains that may be collected, it is a great saving of time and media, if the multiplication of similar strains may be avoided without lessening the therapeutic value of the serum.

Three methods at once suggested themselves—agglutination, opsonic tests, and complement fixation. The agglutination method proved unsatisfactory. Not only did the strains differ greatly, some agglutinating spontaneously, others at only very low dilutions, even with very active immune serum, but the same strains varied markedly at different times, even under conditions that were, as nearly as we could make them, exactly similar.

We tried both microscopic and macroscopic agglutination tests at icebox, room and incubator temperature, finally settling on the macroscopic method at incubator temperature. The cultures were grown on Thallmann agar for twenty-four hours and washed off with normal saline. While we spent considerable time in studying agglutination, especially in connection with our

standardization tests, we did very little work in titrating strains against homologous sera as the great variability in agglutination encountered by other observers as well as ourselves, showed very conclusively the impossibility of using this method in differentiating strains from each other where relatively slight but constant differences must be depended upon to establish groups. Of 30 strains tested, 8 showed no agglutination with normal serum. The others varied from 1:20 to 1:500 with normal serum, 4 showing as good agglutination with the normal as with a polyvalent immune serum in which all the strains were represented. As a rule, of course, the agglutination with the immune serum was at a much higher dilution than with the normal serum. With the immune serum, the agglutination varied from 1:20 to 1:500. The agglutinability varied markedly from day to day; nevertheless, strain 2, which varied rather less than the other strains, would one day give an agglutination of 1:200 and on another 1:100 with the same serum.

The work in agglutination with the meningococcus has been done mainly in an effort to differentiate this organism from closely allied gram-negative strains, and to a less degree for the purpose of standardizing anti-meningitis serum.

Goodwin and von Sholly (2) in 1906 found this method fairly satisfactory for differentiating the meningococcus from other gram-negative cocci in the nasal-pharynx. Absorption tests gave even better results. They say, however, "There was a great difference in the degree of agglutinability of the cultures on different days which made it very difficult to compare results quantitatively." Kolle and Wassermann (3), Krumbein and Schatiloff (4), Columbo (5), Baecher and Hachla (6), Blumenthal (7), and Arkwright (8) report unsatisfactory results with the agglutination tests in identifying the meningococcus. Kutscher (9), Dopter (10 and 11) and Wollstein (12) report fairly satisfactory results in the differentiation of the meningococcus from other organisms. Elser and Huntoon (13) while finding agglutination useful in many cases in identifying the meningococcus, say, "The relatively large number of inagglutinable strains encountered by other observers and ourselves render this method

unfit for purposes of identification and differentiation." They also comment on the great variability of the reaction. "The agglutination tests made with the same strains and the same sera on different days failed to yield uniform results although the organisms were grown on the same lot of medium and under practically identical conditions." Kutscher found more satisfactory results at 55°C. than at 37°C., Wollstein's tests were made at 55°C., Elser and Huntoon found no advantage in using a temperature of 55°C. over that of 37°C.

Opsonic tests were also unsatisfactory. Few strains of meningococcus are suitable for opsonic work, as most of them either show spontaneous phagocytosis or are so resistant that it is impossible to use them for this purpose. Of 34 strains tested, 25 showed very marked spontaneous phagocytosis, 6 moderate and 3 slight. When these last 9 strains were tested with immune serum, they showed so much resistance to phagocytosis that they were unsuitable. Moreover, even with suitable strains the differences are not well-defined enough to permit of accurate grouping.

Work with complement fixation was begun in the summer of 1911, using filtered autolysate as antigen. While the study was not carried very far, as clinical work interrupted, enough was done to convince us that the different strains tended to arrange themselves in groups.

Literature on complement fixation contains little concerning the grouping of meningococcus strains. Citron (14) has referred to the application of complement fixation for the diagnosis of epidemic meningitis by Bruch. Citron (14), Kolle and Wassermann (3), Wassermann and Leuchs (15), Baecher and Hachla (6) and Blumenthal (7) have reported on the use of complement fixation in the standardization of therapeutic immune serum. Others have used the test as a means of differentiating the meningococcus from other gram-negative organisms. Krumbein and Schatilloff (4) found the test specific, i.e., that crossed fixation did not take place with other gram-negative organisms, also that the amboceptors did not run parallel with the agglutinins. Kraus and Baecher in 1909 found meningo-

coccus serum specific with the meningococcus antigen but that not every strain makes a suitable antigen and suggested that there is probably multiplicity of complement binding bodies. Baecher and Hachla in 1910 repeated this suggestion of the multiplicity of complement binding bodies but made no effort to divide their strains into groups by it. In differentiating meningococcus from meningococcus-like strains they found that positive results were specific, negative results of no value. Arkwright in 1911 was unable to distinguish meningococcus from gonococcus by complement fixation. Wollstein (12) found complement fixation of value in differentiating the parameningococci from normal meningococci.

It will be seen from the above that while the complement fixation method has been used extensively in titrating serum and to a slight extent for diagnosis, except for work with the para meningococcus no attempt has been made to use it in grouping the strains of meningococcus.

The rabbits for our work were inoculated by 10 intravenous injections of live culture administered every third day. The dose was increased from one-fourth slant to a whole slant washed off with normal saline. The cultures for inoculation were twenty-four hour growths in neutral veal agar.

Antigens for complement fixation tests were prepared as follows:

The stock cultures were carried on ascitic veal agar, neutral to phenolphthalein. From twenty-four hour cultures transplants were made on neutral salt-free veal agar in potato tubes. The twenty-four hour growth on these was taken up with large swabs and transplanted on neutral salt free veal agar in large wide mouthed bottles. The twenty-four hour growth on the bottle was washed off with 5 to 10 cc. of sterile distilled water according to the amount of growth. The suspension was auto-lized at 56°C. for three hours, then passed through paper pulp and a Berkefeld filter.¹ The antigen was put in small bottles and sterilized at 56°C. for one-half hour for three consecutive days.

¹ The Berkefeld filters used for this work were boiled in sterile distilled water until they were neutral to phenolphthalein.

The antigens were made isotonic just before use by the addition of one part 8.5 per cent saline solution to nine parts antigen.

The total volume of the test was 0.5 cc., one-tenth that of the classical Wassermann test. The anti-sheep system was used; cells in a 5 per cent suspension; from one to two units of amboceptor, and guinea pig serum for complement in a 10 per cent dilution. The system was carefully standardized each day by an amboceptor titration. Sensitized cells were used and the readings of antigen and anti-body content titrations were made the next day, after the cells had settled.

The sera were freshly inactivated each day by heating for one half hour at 56°C.

Antigen and serum titrations were performed according to the technique described in Park and Williams *Pathogenic Microorganisms*, 1914, pp. 184-187. The anticomplementary dose of antigen was determined by testing decreasing amounts of antigen (0.4, 0.3, 0.2, 0.1, 0.05 cc.) in various dilutions. The antigen unit, by which is meant the smallest amount of antigen that gives complete inhibition of hemolysis with about two units of homologous immune serum, was obtained by testing decreasing amounts of antigen (0.25, 0.2, 0.15, 0.1, 0.05, 0.025 cc.) with 0.01 cc. of serum. The anticomplementary property of the serum was tested in 0.04 cc. and 0.02 cc. of undiluted serum. The antibody unit, by which is meant the smallest amount of serum that gives complete inhibition of hemolysis with about two units of homologous antigen, was obtained by testing decreasing amounts of serum (0.02, 0.01, and of a 10 per cent dilution 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02, 0.01 cc.) with about 0.1 cc. of antigen.

The work on the differentiation of meningococcus strains was begun with neither antigen nor serum standardized. A polyvalent antigen of all the strains was made and standardized with 0.01 cc. of serum from a horse immunized against all the strains. The polyvalent horse serum was then titrated with one and a half units of this polyvalent antigen and from one and a half to two units of the polyvalent horse serum were used in standardizing all the monovalent antigens. Each monovalent antigen was

titrated in several dilutions to obtain the antigen unit. From one and a half to two units of the monovalent antigen thus standardized were used in standardizing its homologous immune serum. Any antigen so poor that double this amount was anti-complementary was discarded and not used for serum titra-

TABLE 1

Antigens of twenty-two strains titrated against sera of various strains

ANTIGEN STRAINS	IMMUNE SERA OF GROUP I								IMMUNE SERA OF GROUP II						IMMUNE SERA OF IRREGULAR STRAINS			IMMUNE SERA OF ODD STRAINS				
	1	3	4	7	20	22	23	24	28	10	11	13	14	16	18	2	12	27	6	29	15	17
1	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
8	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
19	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
20	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
21	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
23	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
26	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
28		+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
34		+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	+	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	±	-	-	-	-
10	-	+	-	-	-	-	±	-	+	+	+	+	+	+	-	-	-	±	-	-	±	-
16	-	+	-	-	-	-	-	±	+	+	+	+	+	+	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	+	-	-	-	-
32	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-
2	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-
27		+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+

+ Denotes complete inhibition of hemolysis in as small an amount of antigen with heterologous as with homologous serum.

± Denotes complete inhibition of hemolysis, but not in so small an amount of antigen with heterologous as with homologous serum.

- Denotes incomplete or no inhibition of hemolysis.

tions. Serum was also titrated in a sufficient number of dilutions (1 in 10 and 1 in 100) to determine the anti-body unit. From one and a half to two units of serum (diluted so that 0.01 cc. contained that amount) were used in making the antigen

TABLE 2

Immune sera of twenty-four strains titrated against various antigens

SERUM STRAINS	ANTIGENS OF GROUP I										ANTIGENS OF GROUP II						ANTI-GENS OF IRREGU-LAR STRAINS			ANTI-GENS OF ODD STRAINS			
	1	3	4	7	8	19	20	22	23	24	28	9	10	13	14	16	18	2	12	27	6	29	15
1	+	+		+	+	±	±	+	±	±	±	-	-	-	-	-	±	-	±	-	-	-	-
3	+	+	+	+	±	±	+	+	+	+	+	+	+	+	+	+	±	+	±	+	-	-	-
4	+	+	+	+	±	-	+	+				-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	-	-	+	+	+	+		-	-	-	-	-	-	-	-	-	-	-	-
8	+	±	+		+		+	+				-	±	-	-	-	-	±	±	-	-	-	-
19	+	+	+	+	+		+					+	+		-	-	+	±	-	-	-	-	-
20		+	+	+	+	-	+	+				-	-	-	-	-	-	±	-	-	-	-	-
22	+	+	+	+	±	±		+				±	±	±	±		±	±	±		-	-	-
23	+	+				+	+					±	±	±			±				-	-	-
24	+	+		+		+	+					+	+				±	+	+		-	-	-
28	+	+		±				+				+	+		-	-	+				-	-	-
9	±	±	±	±	±			±	±			+	+	+	+		±	±		-	-	-	-
10	±	+		±	±		-					+	+	+	+	+	±	±	-	±	-	-	-
13	±	±	±	±	±			±				+	+	+			±	±		±	-	-	-
14	±	±	±	±	±		+	±				+	+	+	+	+	±	±	±	+	-	-	-
16	±	±	±	-	-	-		±				+	+			+	+	-	-		-	-	-
18	+	+		+	+	-						+	+			+	+	-	+		-	-	-
2	-	-	-		-		-					-	-		-	-	-	+	-	-	-	-	-
12	±	±	±		±							±	±		-		-	±	+	-	-	-	±
27	±	-	±	-		±						±	±			±	±	-	+	-	-	-	-
6	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	±
29	-	-	-	-		-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	+	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
17	±	±	±	-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-	-	-	+

+ Denotes complete inhibition of hemolysis, in approximately the same amount of serum with homologous and heterologous strains.

± Denotes complete inhibition of hemolysis but not in so small amount of serum with heterologous as with homologous strains.

- Denotes incomplete or no inhibition of hemolysis.

cross titrations. The specificity of the antigens was tested by titrating against a known positive gonococcus serum with practically negative results.

Rabbits were immunized against 40 strains of meningococcus but the cross fixation work was carried out completely with only 29 of these strains. Each antigen and each serum were titrated against all the strains in several dilutions until the smallest amount of both antigen and serum that gave complete inhibition of hemolysis was determined. Table I gives the results of the antigen cross titrations and table 2 the results of the antibody content titrations.

Of the 29 strains of meningococcus tested, 14 fell into one group, 8 into another, 2 cross fixed with each other only by both antigen and serum titrations, 2 did not fix with any other strain and 3 acted irregularly. Strains 1, 3, 4, 7, 8, 19, 20, 21, 22, 23, 24, 26, 28, 34, cross fixed with each other, forming Group I (tables 1 and 2). Strains 9, 10, 11, 13, 14, 16, 18, 32 cross-fixed with each other, forming Group II, but antigens 9, 10, 16 of Group II fixed some sera of Group I. Antigen titrations only of strains 21 and 26 were made against other single strains, as the rabbit sera of these strains lost their anti-body content before serum titrations could be performed. These strains were not included in an antigen made of 6 strains (3, 21, 10, 18, 2 and 12). The sera of these strains (21 and 26), however, gave fixation with this polyvalent antigen and the antigens of these strains fixed all the sera of Group I, hence, we concluded that these strains belonged to Group I. Antigens of strains 6 and 29 gave fixation with their homologous sera only and sera 6 and 29 gave fixation with their homologous antigens only. Antigens and sera of strains 15 and 17 cross fixed with each other. Strains 2, 12 and 27 acted irregularly. In the antigen titrations of these strains cross fixation occurred with the sera of Group I; but the immune sera of strains 2, 12, and 27 fixed complement with their homologous antigens only.

Sera of twelve strains 1, 3, 4, 7, 8, 19, 20, 21, 23, 24, 26, 28 of Group I, seven strains 9, 10, 11, 13, 14, 16, 18 of Group II, the irregular strains 2, 12, 27, the odd strains 6, 29, 15, 17 and nine

unidentified strains 31, 32, 33, 34, 36, 37, 38, 39, 40 were titrated with a polyvalent antigen consisting of strains 3 and 21 of Group I, 10 and 18 of Group II, and the irregular strains 2 and 12. The relationship of strains 6, 15, 17, 27 and 29 had not been determined at the time this antigen was made or they would have been included. These strains alone gave no fixation with the antigen, but our later work demonstrated that they were not related to any of the strains included in the antigen.

An antigen of nine strains consisting of strains 3 and 28 of Group I, 10 and 18 of Group II, 2, 12 and 27, which acted irregularly, 6 and 29 which were not related to any other strains, was made and tested against all the sera that we knew had not lost antibody content and also against the strains that were not included in the cross-fixation work. All sera tested with this antigen gave complete fixation.

As stated above, the monovalent antigens were standardized with the polyvalent immune horse serum for the serum cross titrations, which were made before the antigen cross titrations. We found after we had done the antigen cross titrations, that the antigens gave a much longer range of fixation with their homologous immune rabbit serum than with the polyvalent horse serum. It seems, therefore, that so much antigen was used in the serum cross titrations that a group reaction occurred. This may explain why a more distinct differentiation of strains was obtained by means of antigen titrations than by means of antibody content titrations.

Complement fixation is such a delicate method of differentiating strains of an organism that it is difficult to obtain absolutely consistent results. The age and anti-body content of a serum, the range of the antigen, the strength of the complement, the interval between the completion of the test and the reading of it, the temperature of the ice-box, and the frequency of standardization of sera and antigens are factors that greatly influence the test.

Had this piece of work been performed with sera not more than three months old, the results would have been more satisfactory. The differentiation by both anti-body content and antigen titra-

tions was much more distinct with the fresh serum. As the sera became older, many of them lost their specific anti-bodies, while the reaction with the heterologous strains remained about the same, e.g., the anti-body unit of immune rabbit serum of strain 14 (rabbit No. 814) one month after the rabbit was bled was 0.0005 cc.; with the heterologous antigens the unit was 0.004 cc. Ten months after date of bleeding the anti-body unit of this serum was 0.004 cc. with both homologous and heterologous strains and the serum was useless for differentiation purposes. It is equally difficult to work with serum and antigen of low titre for they seem to give more non-specific reactions than those of high titre. To obtain absolutely consistent results, it is also necessary that both serum and antigen should be standardized each day before use, as serum loses anti-body content in time and both antigen and antibody unit vary with different complements. It was impracticable to make such frequent standardizations, as we worked with so many strains. Much better results can be obtained if only 10 or 15 strains are differentiated at a time.

A constant amount of complement should be used in this test. While the standardization of the system by an amboceptor titration is satisfactory for diagnostic tests, we feel sure that in cross titration work better results can be obtained if complement of uniform strength is used.

The readings were nearly all made from eighteen to twenty-four hours after completion of the test, during which time the tests were stored in the ice-box. The reaction was the same whether the readings were made within eighteen or twenty-four hours, while a variation of the temperature of the ice-box affected the test. During hot months it was found inadvisable to do such work and when it was done readings were made immediately after completion of the test, before the cells settled. Tubes not easily read were centrifuged for three minutes.

A study of the history of the strains proved that the clinical history of the case has nothing to do with the grouping (table 3).

Strains causing severe reactions in rabbits—it being necessary to try several rabbits before one could be found that could be immunized—were:

TABLE 3

*History of strains**Group I.*

1. Isadore (Rockefeller).
3. Patpos (Rockefeller).
4. Rosenstein (Rockefeller).
7. Pittsburg (Rockefeller).
8. Glattmann (Rockefeller).
19. Fessenberg, recovered.
20. Da Vito, severe case; chronic hydrocephalus; death.
21. Fleisher, moderate case; recovered.
22. New Rochelle; sent in from New Rochelle; case not seen.
23. John Carmody; severe case; meningococcic meningitis, cleared up; later pneumococcic meningitis developed and child died.
24. Ercolino, mild case; recovered.
26. Coffey, severe case; chronic hydrocephalus and death.
28. Corsair, sent from Philadelphia.
34. Brier, mild case; recovered.

Group II.

9. Harris (Rockefeller).
10. Windsor (Rockefeller).
11. Messner, severe case; recovered, with deafness.
13. Carbonetti (Bessie); severe case; recovered.
14. Schwartz, severe case; died.
16. Vanaria, moderately severe case; recovered.
18. Pereive, fluid sent in from outside.
32. Gardi, died. Did not receive anti-meningitis serum.

Group III.

- An irregular group, closely allied to Group I.
2. Gouveneur (Rockefeller).
 12. Frances, severe case. died; complicated with pneumonia.
 27. Ferranti, moderately severe case; recovered.

Group IV.

- A miscellaneous group, the strains of which do not cross-fix with any other strains. 15 and 17 cross-fix with each other.
6. Andredakis (Rockefeller).
 29. Depalma, seen late; recovered with two injections.
 15. Lindhard, severe case; blindness; chronic hydrocephalus; death.
 17. Burleigh, (?) hospital case.

1, 2, 7, 16, 18 and 24; 1, 7, 24 are in Group I, 16, 18 are in Group II, 2 is in the irregular Group. No relation exists between virulence and grouping. Neither is there any relation of grouping to the length of time that the strains had been isolated. Group I contained oldest and newest strains.

The subject of the parameningococcus has received considerable attention in France for the last few years owing to the work of Dopter (10 and 11), Menetrier (18) and others. This work has been done along both clinical and laboratory lines. Cases suffering from meningitis that did not respond to the ordinary anti-meningitis serum improved under the use of a parameningitis serum prepared by Dopter in 1912. Dopter in 1911 in studying the so-called para meningococcus, found that he could not differentiate these strains from normal meningococcus by using them as antigens with polyvalent horse serum, but that they could be differentiated by the serum of patients, as the sera of patients suffering from meningococcic meningitis did not fix a parameningococcic antigen, and the sera of patients suffering from parameningococcic meningitis did not fix with a meningococcic antigen.

Wollstein (12) in 1914 published a very careful study of the parameningococcus in which she arrived at similar conclusions using the sera of rabbits immunized with single strains of meningococcus and parameningococcus instead of the sera of patients. She concludes, "The parameningococci of Dopter are culturally indistinguishable from true or normal meningococci, but serologically they exhibit differences as regards agglutination, opsonization, and complement deviation."

By the agglutination method strains 7 and 42 are classed by her as para-like strains. She did not give the results of complement fixation with these strains. In our work it was found by complement fixation that strain 7 fell in Group I and strain 42 in the irregular group allied to Group I.

As transplants of two Dopter parameningococcus strains, P. L. and P. M., were kindly given us by Dr. Wollstein, we were enabled to test them against our strains. We found antigens of P. L. to react with homologous serum only, antigens of P. M. to react with the sera of Group II.

Now if the parameningococcus is to be separated from the normal meningococcus by serological reactions and if agglutination and complement fixation yield different results with the same strains, the question is indeed a perplexing one. With the

method used by us, the results yielded by the complement fixation test have been fairly uniform and specific. The tests have been repeated many times and have, on the whole, checked up very satisfactorily. By this method the strains have fallen into various groups, containing a varying number of strains. Shall we call all the strains except those in Group I, para strains or shall we call them simply groups of normal meningococci? The question is purely academic, yet it is of some importance since the employment of the term "para" differentiates the strains more than seems justifiable. It is, perhaps, better therefore to classify them as belonging to a group coordinate with other meningococcus groups.

Wollstein further concludes:

Because of the variations and irregularities of serum reactions existing among otherwise normal strains of meningococci, it does not seem either possible or desirable to separate the parameningococci into a strictly definite class. It appears desirable to consider them as constituting a special strain among meningococci, not, however, wholly consistent in itself.

This conclusion we would heartily endorse.

CONCLUSIONS

By means of complement fixation the meningococcus may be clearly differentiated from allied organisms. A differentiation of individual meningococcus strains is possible by the use of refined technique but the relationship of strains is so close that it is difficult to obtain absolutely clear cut and consistent results. Of the 29 strains studied by us, 14 seem to form one group and 8 a second group. Three seem to be closely related to the first group but have acted so irregularly that they cannot be classed with it, 2 have shown a relationship with each other only and 2 have shown a relationship to no other strain.

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BACTERIAL CULTURES OF HUMAN SPLEENS REMOVED BY SURGICAL OPERATION

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In recent years, considerable evidence has accumulated which proves that bacteria can be cultivated from the unexposed tissues of the body, especially the lymphatic glands of normal men and the usual laboratory animals. In contrast to these findings, it was formerly assumed that the unexposed tissues of the body are sterile. Absolute freedom of the internal viscera from bacteria would constitute a rather remarkable relationship to our bacterial environment. When we consider the intimate contact of bacteria with skin and more especially the mucous membrane of the alimentary tract, it would be rather unexpected that these structures should form an absolute barrier against the entrance of bacteria. Consequently, it is not surprising to find a fairly definite flora present in the interior of the body under normal conditions. The relative ease with which bacteria may be cultivated from normal tissues naturally becomes very significant in the search for the etiologic agent of an infectious disease, especially when we consider how many diseases there are in which the etiologic agent has been established but in which it has not yet been possible to fulfill Koch's postulates. The organisms of the normal lymphatic glands have added greatly to the difficulty of determining the significance of the organism described by Negri and Miereme (1) and by Bunting and Yates (2) in Hodgkin's disease. In the present paper, I wish to report the results of bacterial cultures made from human spleens and to discuss especially an anaerobic bacillus which was found. The work is of interest largely on account of the relationship of this organ-

ism to the bacillus that has recently been described in typhus fever. An article which attracted wide attention in the past year is the report by Plotz (3) and his associates on the etiology of typhus fever. Plotz described very carefully an extremely interesting bacillus and presented evidence regarding its etiologic relationship to typhus fever, more especially by serologic reactions since it was impossible to fulfill Koch's postulates with this organism. In addition to the collection of a large amount of positive evidence, Plotz also carried out an extensive series of control cultures in conditions other than typhus (198 cases) and also in typhus cases after convalescence. The results were very interesting; the bacillus found in the acute stage of typhus fever was not recovered in any of these controls.

In searching for bacteria in the body, under normal conditions it is much easier to obtain them from the lymph glands and the viscera than from the circulating blood. During the past two years, I have had an opportunity of making cultures at the Massachusetts General Hospital, upon spleens removed on the surgical service. The majority of these splenectomies were performed for pernicious anaemia. The routine of the bacteriological examination was as follows. After thoroughly searing part of the surface of the spleen, a fairly large piece of splenic tissue was cut out under aseptic precautions. Small pieces of this, 3 to 5 mm. square, and 2 to 3 and sometimes 5 mm. in thickness, were used for inoculation. The following media were employed, litmus milk, blood agar (using 1 part of agar to 1 part of human blood), citrated blood (using 1 part of human blood to 4 parts of 2 per cent sodium citrate in normal salt solution), bile broth ($\frac{1}{2}$ per cent sodium glycocholate in ordinary bouillon $\frac{1}{2}$ per cent acid), Ficker's brain medium (4), Dorsett's egg medium, and glucose (2 per cent ascitic agar; ordinary agar, 2 per cent glucose agar, and 7 per cent glycerine agar. The media containing blood were incubated aerobically at room temperature for the possible development of protozoa; the cultures remained sterile. The litmus milk, Ficker's and Dorsett's medium and the glucose ascitic agar were incubated both aerobically and anaerobically. Especial attention was given to the litmus milk, usually a dozen

tubes were inoculated, eight of which were incubated anaerobically and four aerobically. The plain agar, the glycerine and glucose agar were used as controls for ordinary contaminations and secondary invaders and were incubated aerobically.

Comparatively few of the spleens proved to be sterile. In the accompanying outline, table 1, I have mentioned only those media upon which growth occurred.

TABLE 1
Showing the results of cultures from spleens

SERIAL NUM- BER	DIAGNOSIS	RESULT
1	Pernicious anemia	Micrococcus from bile tubes
2	Pernicious anemia	Sterile
3	Pernicious anemia	Micrococcus from bile and milk tubes
4	Pernicious anemia	Micrococcus from bile and milk tubes
5	Simple hypertrophy	Sterile
6	Pernicious anemia	Anaerobic bacillus from milk tubes
7	Infantile primary spleno- megaly	Sterile
8	Pernicious anemia	Anaerobic bacillus from milk tubes*
9	Pernicious anemia	Sterile
10	Pernicious anemia	Anaerobic bacillus from milk tubes

* One of the milk tubes also showed a micrococcus.

In all of these cultures, it is noteworthy that no definite diphtheroids were obtained. This result is suggestive of a difference in the flora of the spleen and lymphatic glands.

No growth occurred in any of the tubes of plain agar or of glucose or glycerine agar. None of these were incubated anaerobically, but it is extremely unlikely that the anaerobic bacillus, found in three cases, would have developed since, even upon sub-inoculation, it would not grow upon these agar media without enrichment with body fluids and it was not obtained by direct inoculation from the spleen into ascitic glucose agar containing a piece of tissue (this tissue being furnished by inoculation of a rather large piece of the spleen under examination).

The micrococcus appearing in cases 1, 3 and 4, grew out both aerobically and anaerobically, but only in fluid media; namely,

bile and milk. On subinoculation, however, it grew freely on glucose agar but scantily indeed, upon plain agar. No attempt was made to establish the identity of these three cultures of micrococci to one another. They differed definitely, however, from the ordinary staphylococcus aureus and albus and from six strains of micrococci isolated from the air. Culturally, the most important characteristic was the necessity of using glucose agar to obtain a good growth. Morphologically, the organisms were distinctly pleochromic. This characteristic was seen very well with either methylene blue or Giemsa's stain. The preparations stained with methylene blue, frequently showed small clusters of six or eight organisms, perhaps half of the organisms staining a solid, uniform color, while the others would stain only at the periphery appearing just as blue rings, some faintly and some deeply stained.

Tests for complement fixation were carried out with the micrococcus from Spleen IV, against the sera of cases of pernicious anaemia and normal sera. As further control, antigens were made from a Gram positive coccus obtained from the air and also from the gonococcus, in order to have a representative of the pus-forming cocci. The routine employed for complement fixation was the same as that described later in connection with the work on the anaerobic bacillus.

Complement fixation occurred in four or five cases of pernicious anaemia and complete haemolysis resulted in five control cases. However, the same results with equally strong reactions occurred with the antigens prepared from the coccus from the air, whereas, with the gonococcal antigen, the same cases were positive and the reactions were even strong in two instances. The Wassermann reaction in these cases, using a cholesterinized human heart antigen, was also positive at this time, though previously, it had been repeatedly negative. Specific venereal disease could be excluded with reasonable certainty except for a possible question of syphilis in one of the splenectomized patients. Clinically, the most important feature which might bear upon these results, is that the four cases giving positive fixation reactions, had been splenectomized from four to eight months pre-

viously, whereas, the one case giving a negative reaction had not been splenectomized. Furthermore, upon repeating these tests six weeks later, the reactions were negative in all instances to all of these four antigens. One of the patients, who was subsequently followed for several months, showed reactions that fluctuated from negative to strongly positive.

The anaerobic bacillus occurring in three cases, is of interest, both from its possible causative relationship to pernicious anaemia and from its striking resemblance to the organism found by Plotz in typhus fever. This bacillus appeared only in the tubes of litmus milk, incubated anaerobically. Growth appeared usually in three to five days, though sometimes, it was delayed until the tenth or fourteenth day. The litmus was either acidified or decolorized and the protein was precipitated but without the formation of a clot. Of eight or ten tubes, inoculated in this way from each case, there were always two or three in which no growth could be found. Usually, no change occurred in the milk unless bacterial growth developed. In some instances, the splenic tissue caused a precipitate of the proteins but without acidification. After a month, negative cultures were discarded.

Of all of the aerobic cultures of litmus milk, there was but one tube showing the development of the bacillus that usually appeared only in the anaerobic cultures. On subinoculation, no growth could be obtained except under anaerobic conditions equally as strict as those required by the cultures developing from this same spleen in the tubes placed in an anaerobic jar. Consequently, although the tube was not placed in an anaerobic jar, it does not seem possible that this development occurred under aerobic conditions. Theobald Smith (5) at the time that he introduced this method, emphasized the possibility of obtaining a high grade of anaerobic conditions in an open tube by the use of a piece of tissue. In considering the frequency with which this organism occurred in the cases of pernicious anaemia, there is one feature that should be emphasized. The examination of the cases in table 1 covered a period of two years with an interim of eight months between cases 5 and 6, the first anaerobic bacillus being found in case 6. Although the same routine examina-

tion was followed throughout all the entire period, still it is not unlikely that this bacillus was overlooked as it was not searched for specifically. Consequently, neither the positive nor the negative cases from 1 to 5 inclusive, are of quite the same value as the subsequent ones. Of the five cases, then, in which systematic examination was carried out for this bacillus, it was found in three of four cases of pernicious anaemia, one case of pernicious anaemia and one control case being negative.

Unfortunately, I obtained no data in regard to the relative numbers of these organisms in the spleen; consequently, there is no evidence at hand to determine whether this organism had colonized and was multiplying or whether it was merely filtered out from the blood by the spleen. The estimation of the number of these organisms in the spleen is not easy, for it grows with difficulty even on subinoculation and in the original isolation from the spleen, it has so far grown only in fluid media. Although a certain proportion, about one-fourth, of the tubes of milk remained sterile even when inoculated with large pieces of tissue, still it is entirely possible that the organisms may have been present in fairly large numbers. It is not unlikely that only a small proportion of the organisms develop, for the splenic tissue while providing excellent anaerobic conditions may, nevertheless, have restrained the growth of these bacteria. There is perhaps, some indication for this view in the fact that subcultures grew readily on glucose ascitic agar but inoculations of infected splenic tissue failed to give growths on this medium. Another illustration of the restraining influence of body fluids was found in the use of hydrocele fluid of high specific gravity (1.025). Growth took place much more readily when a few drops ($\frac{1}{2}$ cc.) of this fluid were used with 20 cc. of glucose agar, than when large amounts (7 cc.) were employed. With the larger quantity, good growth took place though the first appearance of growth was restrained for several days (three to five) after it had appeared in the tubes containing only a little hydrocele fluid. Tissues fixed in Zenker's fluid and stained by the Gram-Weigert method did not show any definite micro-organisms.

Cultures of the bacilli from Spleens VI, VIII and X showed

no pathogenicity for lower animals. Rabbits were inoculated intravenously and mice, rats and guinea pigs were inoculated intraperitoneally without the production of any noticeable effect.

DESCRIPTION OF ANAEROBIC BACILLUS

Morphology and staining properties

In young cultures, this organism is a small short bacillus, moderately blunt at the ends and non-motile, measuring usually 1 to 1.5 microns in length. The individual organisms do not vary much in size and shape until the cultures become old. Then distinct pleomorphism appears; many coccoid forms are found and occasionally some forms appear that slightly suggest the diphtheroid group. Young cultures are stained readily and uniformly with the ordinary aniline dyes and very intensely with Gram's stain. Smears from cultures were stained for three minutes with anilin-gentian violet and, without rinsing in water, were washed for one minute with Gram's iodine; they did not decolorize after fifteen minutes washing in 95 per cent alcohol.

Cultural characteristics

The organism requires anaerobic conditions for its growth and develops best on media that have been enriched with body fluids or tissues. No growth occurs, either aerobically or anaerobically on ordinary litmus milk or on broth or agar, either plain or with the addition of glucose or glycerine. Excellent growth occurs in tubes of litmus milk containing a piece of tissue (rabbit kidney or spleen) and placed in an anaerobic jar, or in deep glucose ascitic agar tubes even without any further measures for removing the oxygen. Growth in these tubes extends upward to within 2 cc. of the surface, without gas production, but with the formation of acid and intense clouding from the precipitation of the proteins in the medium. No growth was obtained on the blood agar using the triple N medium of Novy, MacNeal and Nicolle, substituting human for rabbit's blood. On Dorsett's egg medium, a scanty growth occurred which soon died out on subinoculation. A strain of the Plotz bacillus behaved in a

similar manner on Dorsett's medium. This bacillus, therefore, in its morphology, its staining reactions and in its cardinal cultural characteristics does not differ from the organism discovered by Plotz in typhus fever.

The anaerobe from spleen X was killed by heating for fifteen minutes at 55° C.

The next point that was taken up was the significance of these anaerobic cultures from the spleens of cases of pernicious anaemia. As a preliminary step, it was, of course, necessary to determine the relationship of these three strains to each other. The relationship to the Plotz bacillus, however, is a much more interesting feature and also offers valuable information in establishing the position of these cultures from the spleen. Accordingly, a detailed comparison was made of these three strains of anaerobes with each other and with the typhus organism both in regard to their fermentation of various carbohydrates and in regard to their serological reactions.

In the absence of Dr. Plotz in Serbia, Dr. Libman of the Mount Sinai Hospital in New York, very kindly furnished a strain of the Plotz organism. In his report on typhus fever, Plotz and his associates described in detail, the behavior of his organism on various carbohydrates and its serological reactions. Accordingly, I have used these same carbohydrates and have performed serological reactions with human blood serum in a variety of diseases and with the serum of rabbits immunized against these organisms.

The cultures on carbohydrates were carried out as described by Plotz 3 per cent agar faintly tinted with litmus being used, with an acidity of 1 per cent N acid to phenolphthaleïn, with 2 per cent of carbohydrate and with one part of ascitic fluid to three parts of agar. The cultures were incubated usually for six days and occasionally for eight days when the growth was late in appearing. Unfortunately, with the same strain of bacillus and the same carbohydrate, the results, in some instances, varied somewhat. It seems that the result depended, in part, upon the luxuriance of growth that was obtained and this, in turn, varied with different specimens of ascitic and hydrocele

fluid. Some difficulty was experienced in making up 2 per cent of inulin in 3 per cent agar. The inulin was made into a thin paste in cold water and this was stirred gradually into the hot agar (100°C.). Solution appeared to be complete but a precipitate subsequently occurred during sterilization.

The fermentation tests showed that one of these bacilli from pernicious anaemia differs slightly from the other two and that none of the three coincide fully with the Plotz organism in their behavior on carbohydrates. Of the ten that were tested, production of gas did not occur in any instance. Fermentation—with the production of acid in sufficient amount to cause precipitation of the protein in the medium occurred in the following instances, glucose, maltose, galactose, and mannite, and occasionally a faint precipitate with lactose. The strain of culture from Spleen VI gave a faintly acid reaction in three of nine cultures on lactose. The fermentation of mannite constitutes a definite exception to the fermentation reactions of the Plotz bacillus. The cultures from Spleen VI and Spleen VIII grew well on mannite and fermented it rather easily with the production of acid and a well-marked precipitation of protein. With lactose, however, there was not much difference between these organisms and the bacillus found in typhus fever. The fermentation of this sugar was, at most, very slight and usually it did not occur at all. In this connection, it is of interest that although Plotz found no fermentation by any strains of his organisms growing on litmus lactose ascitic agar, still, slight fermentation occurred in litmus milk. This, however, does not necessarily mean that the lactose of the milk was fermented, since Plotz' organism ferments galactose very readily. One possible source of galactose in milk would be the splitting of the lactose by slight bacterial fermentation before sterilization of the milk was effected. In this connection, it should be noted that *B. typhosus* does not split lactose but does slightly acidify litmus milk.

Turning to the carbohydrates that are not fermented by the bacilli from the spleen, we have the following: saccharose, arabinose, raffinose, inulin and, in most instances, lactose.

Of this list, it should be mentioned that growth was secured on all these carbohydrates although no fermentation resulted. Considerable difficulty was experienced in obtaining growth on raffinose, arabinose and inulin and, at best, the growth was always scanty. Growth took place readily on saccharose and not much difficulty was experienced with lactose or dextrine. The three strains of bacilli from the spleen agree rather closely with each other and their principal difference from the typhus bacillus lies in their reactions on inulin and mannite, the typhus organism fermenting inulin and not mannite while the reverse is true with two of these cultures from the spleen. However, the strain from Spleen X does not ferment mannite and differs from the Plotz' bacillus only in its failure to ferment inulin. In this connection, I wish to explain that the technique of the proper preparation of inulin and the cultivation of the Plotz' organism on it was very difficult for me. In fact, the typhus organism is extremely capricious in its growth and Plotz deserves great credit for establishing a practical method for its cultivation. But even when working with a strain of the typhus organism, I was unable to secure clear cut fermentation of inulin.

None of these cultures from the spleen coincide exactly in their growth on carbohydrates with the typhus bacillus. The differences, however, are not greater than occur, for example, in different members of the species *B. diphtheriae* or *B. dysenteriae*. So far as the fermentation tests go, then, if we interpret them in the light of the findings in diphtheria and bacillary dysentery, we must conclude that these organisms from pernicious anaemia and from typhus fever are very intimately related members of a single group of bacteria—that they are varieties of a single species.

In the serological work, agglutinations and complement fixation tests were performed. In carrying out the agglutination tests, one is at a disadvantage on account of the tendency that some of these cultures show to clump spontaneously in salt solution without the addition of serum. In view of this difficulty, only a few agglutination tests were performed. My attention has recently been called to a method by which this difficulty can

probably be overcome. In working with bubonic plague Strong and Teague (6) found that $\frac{1}{10}$ per cent of sodium chloride would prevent this spontaneous clumping and still furnish sufficient salt to allow specific agglutination.

The following routine was observed in the serological work. For agglutination tests, a young culture five days old, on glucose ascitic agar was used. Olitsky recommends the microscopic method, carrying out the test very rapidly, before spontaneous agglutination can occur in the controls in salt solution, preferably within one hour. With the necessity of observing this time limit, I found it rather difficult to secure satisfactory readings when working with an extensive series of preparations. One strain of the organism from pernicious anaemia, case 6, was found which did not slump excessively in salt solution. A very thin suspension was prepared and macroscopic tests were performed. The preparations were left at room temperature for an hour and were read after they had stood over night in the refrigerator.

In the preparation of the antigen for complement fixation, the digestion of the bacterial suspension in distilled water, was carried out at 37°C. for periods of ten to fourteen days. This long period seemed necessary for even a moderate breaking up of the strongly Gram-positive bacilli. Moreover, instead of filtration through a Berkefeld filter, the suspension, after digestion, was centrifuged for a short time, at low speed. The supernatant fluid was distinctly opalescent and, on standing, it tended to settle out only very slowly indeed. Before use, it was of course made isotonic with salt solution. This method of preparation of bacterial antigens has, in general, yielded products of high antigenic value and comparatively low anticomplementary power, in contrast with the short digestion and filtration through a Berkefeld candle as advised by Olitsky. The usual standard procedures were followed in the tests for complement fixation. In the titration of the antigens for anticomplementary power, one-half the amount that just permitted complete hemolysis was usually employed. In some special cases, the fixing power of the antigen was so strong that the dose was reduced to one-fourth of the maximal amount that allowed complete haemolysis.

The customary sheep-cell, rabbit-serum haemolytic system with guinea pig complement was employed, two haemolytic units being used. Of the serum to be tested, 0.1 cc., undiluted, was employed in a total volume of 2.2 cc. In the selection of rabbits for immunization, it is extremely important to select individual animals that do not exhibit non-specific complement fixation. Not infrequently, individual normal animals occur that give positive reactions with both the Wassermann and the gonococcal antigens, reactions which, in the human, are in no way interdependent. It was found that animals giving this non-specific fixation in the Wassermann and the gonococcus tests, also fixed complement with the Plotz organism and with the bacillus from the spleens. Fortunately, Kolmer and Trist (7) have found that the reaction in normal animals tends to be constant and does not fluctuate from positive to negative. My own experience agrees with this. Accordingly, at the beginning of the experiment, animals were selected for immunization and for normal controls whose fixation reactions were negative to these antigens. Complement fixation tests on the bacilli from the cases of pernicious anaemia against the serum of rabbits immunized to these organisms, showed definite cross-fixation. The accompanying outline gives the results for either an antigen or an immune serum of each of the three strains of anaerobes from pernicious anaemia.

Degree of fixation

RABBIT SERUM IMMUNE TO BACILLUS FROM		ANTIGEN FROM SPLEEN VI	BACILLUS FROM SPLEEN VIII
Spleen VI.....	{ 1	Complete	None
	{ 2	Complete	Complete
Spleen X.....	{ 3	Complete	Complete
	{ 4	Complete	Complete
Normal rabbits.....	{ A	None	None
	{ B	None	Trace
	{ C	None	Trace

Complete, No haemolysis. None, Complete haemolysis. Trace, Almost complete haemolysis.

For comparison of the Plotz bacillus with these anaerobes, some preliminary fixation reactions were carried out by testing an antigen from the organisms from Spleen VI against a corresponding immune rabbit serum, an immune serum from a rabbit injected with the Plotz organism and against a variety of human sera. In order to guard against the occurrence of very general non-specific fixation, the reactions to a Wassermann antigen (cholesterinized human heart) are included. A corresponding set of agglutination reactions were carried out with this bacillus and these sera. The results show both cross-fixation and cross-agglutination. The data are given in table 2.

These results were confirmed on a larger scale, antigens being used that had been made from bacilli isolated from two of the spleens (cases 6 and 8) and from the Plotz bacillus. These were tested against patients and against rabbits immune to the Plotz bacillus and to two of the strains of organisms obtained from the spleens (cases 7 and 10). For these tests, one of the rabbits immunized against the organism from Spleen VI, had died suddenly. In its place, a rabbit was substituted that had been injected four months previously with this strain of organism.

The results are of interest since cross fixation occurs freely and practically constantly between the various sera and antigens. There is only one apparent exception and this occurs in the animal that had not been injected for a long period. Its immunity was evidently not of high grade, since absolutely complete fixation was not obtained with its own antigen and it failed to cross fix with the other sera. A duplicate animal immunized more recently than this one, did give cross fixations. Although the serum of this animal, which had not been recently injected, failed to give cross-fixation, yet the antigen gave cross-fixation with the sera of other animals immunized to the organism from Spleen X.

The results of these fixation tests furnish definite evidence of a close relationship between these bacilli and do not supply evidence of any difference between them.

The data are given in table 3.

TABLE 2

Complement fixation and agglutination with organism from Spleen VI

COMPLEMENT FIXATION				AGGLUTINATION OF BACILLUS FROM SPLEEN VI	
Immune to		Antigen from organism of Spleen VI	Wassermann	Dilution of serum	
				1-50	1-100
Organism from Spleen VI.....	1	Complete	None	Partial	None
	2	Complete	None	Complete	Complete
	5	Complete	None	Complete	Complete
Plotz' organism.....	6	Complete	None	Complete	Complete
Normal.....	A	None	None	None	None
	B	None	None	None	None
	C	None	None	None	None
	D	None	None	None	None
	E	Almost complete	Almost complete	Partial	None

Patients' serum

Diagnosis:					
Pernicious anemia...	1	Trace	Partial	None	None
	2	Trace	Complete	None	None
	3	Trace	None	None	None
	4	Trace	None	Well marked	None
	5	None	None	Well marked	None
Secondary anemia...	6	Complete	None	Well marked	None
	7	Partial	None	None	None
	8	Trace	None	Well marked	None
Cerebrospinal syphilis.	9	Almost complete	Partial	None	None
Tabes.....	10	Complete	Partial	None	Well marked
Endocarditis.....	11	Trace	None	None	None
Lobar pneumonia.....	12	Partial	None	None	None
Aortitis.....	13	Complete	None	Well marked	None
Peritonsillar abscess...	14	Complete	None	Complete	Complete
Gastric ulcer.....	15	Trace	None	Partial	None
Myasthema gravis....	16	Complete	None	Well marked	None
Visceroptosis.....	17	Trace	None	Well marked	None
Hyperthyroidism.....	18	None	None		

TABLE 3

Showing cross fixation tests with rabbit sera toward antigens of the Plotz' bacillus and the bacillus from pernicious anemia

IMMUNE SERA FOR ORGANISMS OF		DEGREE OF FIXATION		
		Antigens		
		Spleen VI	Spleen VIII	Plotz' bacillus
Spleen VI.....	1	Almost complete	Complete	Complete
	2	Almost complete	Almost complete	Complete
Spleen X.....	3	Complete	Complete	Complete
	4	Complete	Complete	Complete
Plotz'.....	5	Complete	None	None
	6	Complete	Complete	Complete
Normal rabbit sera.....	A	None	None	None
	B	None	Trace	Trace
	C	None	Trace	Partial

The following fixation tests were carried out with the organism from Spleen VIII and with the Plotz bacillus against human sera three cases of pernicious anaemia and five control sera being used. The results are given in table 4.

TABLE 4

Showing fixation with serum of pernicious anemia and control cases

CASE NUMBER	DIAGNOSIS	DEGREE OF FIXATION		
		Antigen		
		Bacillus Spleen VIII	Plotz' bacillus	Wassermann
1	Pernicious anemia.....	None	None	None
2	Pernicious anemia.....	Complete	Complete	None
3	Pernicious anemia.....	None	None	None
4	Cholangitis.....	Complete	Complete	Complete
5	Gastric neurosis.....	None	None	None
6	Arterio-sclerosis.....	None	None	None
7	Gastric neurosis.....	None	None	None
8	Mitral insufficiency.....	None	None	None

Both the antigens of the bacillus of Plotz and of Spleen VIII were used in one-fourth the dose that just failed to cause inhibition of haemolysis.

Complete fixation in two out of eight cases tested against the Plotz antigen seemed rather unexpected, especially as the anti-

gen was used in only half the quantity that was permissible from the stand point of its anticomplementary power. Seventeen additional patients were tested to determine whether complete fixation is as frequent as these results indicate and in these cases complete haemolysis occurred in all except three; in these the haemolysis was only partial. The fact that complement fixation may be obtained with this antigen in diseases other than typhus fever, does not impair the value of the fixation tests reported by Olitsky in typhus cases. Obviously, in diagnosing a disease by complement fixation tests, it is necessary, first of all, to reduce the quantity of antigen or otherwise to adjust the conditions so that no normal sera will give positive reactions. While it is important to know that non-typhus cases will fix complement rather readily with this antigen, the striking feature in these results is that the reactions of the bacillus from the spleen coincide precisely with those of the Plotz bacillus. This is further indication of some biological relationship between these organisms.

INTERPRETATION OF RESULTS

It is evident that there is a close relationship in the essential characteristics of this bacillus cultivated from the spleen and the bacillus isolated by Plotz. The interpretation that suggests itself most readily is that these organisms are normal inhabitants of the body and that although they are highly parasitic, they are not pathogenic. The slight difference in the fermentation of sugars does not militate against the assumption that these bacilli from pernicious anaemia and typhus fever belong essentially to one species. It will be remembered that Plotz found the various strains of his organism to be entirely constant in their fermentation reactions. Nevertheless, it does not seem improbable that a more extended series would reveal some variation. If this bacillus from the spleen should develop in cultures made for the diagnosis of typhus fever, it could not be distinguished from the Plotz bacillus except by a detailed study of its reaction on carbohydrates. The small series of agglutination tests indicate the close relationship of the bacillus from the spleens to the

Plotz bacillus, and the complement fixation tests are in agreement with this conclusion. However, complement fixation can at times be the least specific of the immunity reactions. Failure to show cross fixation would speak strongly in favor of distinct species, whereas positive results do not merit so much significance.

The finding of the Plotz bacillus in Brill's disease has been advanced as additional evidence of the identity of this disease and typhus fever, but this argument loses its significance more or less completely when this same bacillus is found in an entirely different disease.

If we consider the bacillus described by Plotz to be the cause of typhus fever, then it would be conceivable that this anaerobic organism from these spleens might be the cause of pernicious anaemia. Although widely different parasites may produce almost identical symptoms, we do not have any well established instances in which organisms that are closely related biologically, produce widely divergent clinical effects. One example which suggests itself is the skin lesions of *B. leprae* and *B. tuberculosis*. However, on closer examination, the skin lesions caused by the two organisms from the standpoint of histology are closely related. Another example for consideration, is the skin lesion produced by *Leishmania tropica* and kala azar produced by *L. donovani*, but in this case the differentiation of these forms into two species is now being called into question. With the limited amount of data at hand, it is difficult absolutely to exclude this bacillus, isolated from cases of pernicious anaemia, as the possible etiologic factor of the disease, with definite proof that would meet all theoretical objections; at least, this is true when we consider the number of diseases in which the etiologic factor is established without fulfilling Koch's postulates and the widely divergent bases upon which investigators in different fields propose new etiologic organisms. Perhaps crucial evidence would be obtained by the examination of normal spleens. The bacteriological study of the spleen is not very satisfactory except under special conditions. It seemed worth while to report the results of the cultures from these spleens without waiting for controls from

other conditions, since surgical removal of the spleen is rather uncommon. However, I should be very much surprised if subsequent examination showed the bacillus found in pernicious anaemia to be specific for this disease and that it did not occur in other conditions. Nevertheless, except for the knowledge of the Plotz organism and its relation to typhus fever, I am sure that it would be much more difficult for me to dissuade myself of a possible etiologic rôle of this organism in pernicious anaemia.

The simplest interpretation of these results is that the bacillus isolated from the spleen of cases of pernicious anaemia and from the blood of cases of typhus fever is merely a part of the normal flora of the body. Such organisms might gain a better foothold during the course of a serious disease especially in a severe infection or pronounced anaemia. There are several suggestive points that support this view. It is significant that antigens prepared from the bacillus isolated from the spleen, fix complement indifferently with cases of pernicious anaemia and with other diseases. The isolation of organisms evidently belonging to the same species from such widely different conditions as pernicious anaemia and typhus fever, constitutes extremely strong evidence that they are not the causative agent of either disease. On the other hand, it must be kept in mind that in a long series of blood cultures in control cases, Plotz, in no instance, found the organism which occurred so frequently in typhus fever. Furthermore, Olitzsky found that complement fixation gave positive reactions in a high percentage of typhus cases under conditions in which non-typhus cases reacted negatively.

Plotz has evidently discovered a new organism or a new group of organisms, which are highly parasitic. They are non-pathogenic for lower animals and probably non-pathogenic for man. The recognition of this anaerobic organism constitutes an important advance in bacteriology. I would like to think that a new etiologic agent had been discovered. However, I am not prepared to accept that either the anaerobic bacillus isolated from typhus fever or from the spleen of cases of pernicious anaemia is the specific etiologic agent in either disease.

CONCLUSIONS

1. Bacterial cultures were made of human spleens removed for surgical purposes in ten cases. The cultures from four of these spleens remained sterile. In three instances, a micrococcus was obtained and in three other cases an anaerobic bacillus developed.

2. The anaerobic bacillus in its morphology, in its cultural characteristics and in its serological reactions resembled very closely the bacillus isolated by Plotz in typhus fever. The evidence which is at present available, indicates that these anaerobic bacilli, isolated by Plotz from typhus fever and appearing also in cases of pernicious anaemia, constitute essentially a single species.

3. The weight of evidence indicates that this group of organisms represents parasitic but non-pathogenic flora of the human body. There is not sufficient evidence to ascribe a specific etiologic rôle to members of this group of anaerobic bacilli in either typhus fever or in pernicious anaemia.

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SCIENTIFIC PROCEEDINGS OF THE SOCIETY FOR
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1. AGGLUTINABILITY OF BLOOD AND AGAR STRAINS OF TYPHOID BACILLI

Carroll G. Bull, and Ida W. Pritchett: Gay and Claypole found that two generations on 10 per cent rabbit blood agar rendered typhoid bacilli inagglutinable to an immune serum produced by a plain agar strain, but that a serum produced by a blood strain agglutinated equally well both blood agar and plain agar strains.¹ These authors observed also that a blood strain immune serum agglutinated freshly isolated inagglutinable strains, and they were thus able to identify such strains without first growing them for a number of generations on artificial media.

In contemplating the production of a general agglutinating serum according to the above method, we first attempted to render our laboratory strains inagglutinable by growing them on 10 per cent rabbit blood agar. Two generations on blood agar failed to change the agglutinability of these strains. Cultivation on blood agar was continued and the agglutinability tested with an agar strain immune serum after each two or three successive transfers. No difference between the agar and blood strains could be detected even after twenty-five generations on blood agar. We then collected fifty-five strains from various sources and subjected them to a similar procedure. Twenty of the collected strains were in the second generation after isolation from patients. The individual strains varied a good deal in agglutinability, but the corresponding blood and agar strains ran quite parallel.

One inagglutinable strain was encountered. The plain agar culture was as inagglutinable as the blood strain. This strain did not produce acid in maltose, mannite, and galactose. It produced indol as readily as coli bacilli, and was very toxic for rabbits; 1/150 of an agar slant per kilo of body weight killed rabbits within from three to twenty-four hours. Both agar and blood strains were agglutinated by a homologous serum produced with the agar strain. This serum agglutinated other typical strains, but only 1/5 as well as the homologous strains. A serum produced with a typical strain protected rabbits passively against three lethal doses of the irregular strain.

Another method was used to establish the identity of this strain. Rabbits actively immune to typhoid bacilli are very resistant to intoxication by these organisms; they withstand from thirty to forty lethal doses. Since passive protection is limited to a few lethal doses, active

¹Gay, F. P. and Claypole, E. J., Archives of Internal Med., 1913, xii, 621.

protection can be used to advantage in identifying and classifying such organisms. In this particular case, rabbits immunized with typical typhoid bacilli were only slightly intoxicated with seventy-five lethal doses of the irregular strain. Hence, we are dealing with a true typhoid bacillus or active protection against such organisms is quite non-specific.

Conclusions. Growth on 10 per cent rabbit blood agar did not affect the agglutinability of fifty-eight strains of typhoid bacilli.

An organism isolated from a typhoid patient showed irregularities in agglutination, sugar fermentation, and indol production, but was identified as a typhoid bacillus through active protection tests.

2. A SEROLOGICAL STUDY OF ETHYLHYDROCUPREIN (OPTOCHIN) IN ACUTE LOBAR PNEUMONIA

Henry F. Moore and Allan M. Chesney: Ethylhydrocuprein (optochin), a derivative of hydroquinine, has a specific inhibitory and bactericidal action on the four main groups of pneumococci in the test tube, and confers a protection on mice against several lethal doses of these microorganisms. It was shown that the serum of animals and man receiving this substance acquired an inhibitory and bactericidal action on the pneumococci.

The present study is concerned with this acquired bactericidal action upon pneumococci of the serum of patients suffering from acute lobar pneumonia after administration of the drug. The "plate" method was used. The hydrochloride of the drug was used, and given by mouth in order to obtain comparable results; the various samples of serum which were obtained both before and during the treatment, were studied at the same time. To avoid agglutination, due to a developing immunity on the part of the patient, simulating a bactericidal action, a strain of pneumococcus belonging to a group other than that of the infecting pneumococcus was generally used; wherever this was not the case, agglutination tests were made to rule out this fallacy.

It was established that the dosage of the drug could be so regulated that decided bactericidal action appeared in the serum within a few hours after administration of the first dose of the drug, and that this action remained more or less constant throughout the treatment. It was sought to obtain a rapid appearance of this action in the serum, and to maintain it at as constant a level as possible in view of the fact that pneumococci, when in contact with a concentration of the drug lower than that required to produce a bactericidal action, become "fast" or resistant to it in a relatively short space of time; and, also, because the pneumococci are killed only after several hours contact with the drug, the time required depending not only on the concentration of the optochin, but, to a large extent, on the rate of growth of the bacteria. Owing to the fact that toxic symptoms, which resemble those of quinine poisoning and which are rarely serious, may appear as a consequence of giving too large amounts, a limit was set to the quantity given per twenty-four hours. The dosage recommended for an

average sized individual is 1.5 gram per twenty-four hours, divided as follows: an initial dose of 0.45 gram, and 0.15 gram every third hour thereafter, up to 1.5 gram, for the first twenty-four hours; for each following period of twenty-four hours, 0.15 gram every two and one-half hours. It is advised that the amount of drug should be given in relation to body weight, so that adult patients get, on an average, from 0.024 to 0.026 gram per kilogram of body weight per twenty-four hours. In every case in which this relation obtained and in which the doses were suitably spaced, the serum showed a strong bactericidal action on the pneumococci.

3. HUMAN SENSITIZATIONS

Robert A. Cooke: (See this number, p. 201.)

STUDIES ON ANTIBACTERIAL SERUMS

I. STANDARDIZATION OF ANTIMENINGITIS SERUM

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Antimeningococcus serum has proved to be an excellent therapeutic agent in the majority of cases of cerebrospinal meningitis in which it has been employed. There have been, however, a smaller number of cases in which the serum produced little, if any, beneficial influence upon the course of the disease. It is greatly to be regretted that, in the reports of the use of the serum so little has been known, or recorded, of its potency. This lack of data is due to the fact that no uniform method of estimating efficiency has been employed by the laboratories producing the serum.

It is evident, *per se*, that a serum which exerts a curative influence upon a case of meningitis, possesses properties counteracting the infecting organism. Conversely, a serum that does not produce a change in the course of the disease, does not possess enough antibodies to overcome the infecting organism. This absence of effect, may be due to a lack of polyvalency or to insufficient potency. The fact that a serum can possess therapeutic value makes it incumbent upon the producers of the serum to determine its effect upon any strains of meningococci. In other words, the serum must be standardized as to potency and polyvalency.

MENINGOCOCCUS TYPES

In order to understand fully the problem of standardization of antimeningococcus serum, some facts in the biology of the meningococcus must be considered. The meningococci do not compose a homogeneous group, but have been found by various

investigators (Dopter (1), Wollstein (2)) to consist roughly of two main groups, the true meningococci and the parameningococci. Wollstein, however, finds that the true meningococci include many atypical strains. These efforts at classification have been made upon the basis of agglutination and complement fixation reactions. As in all other groups of bacteria, a variable susceptibility to these reactions has been observed among different strains. The inadequacy of these reactions for drawing lines of classification is emphasized by the fact that a serum immune to the gonococcus will give positive reactions with the meningococcus-Wollstein (3).

PATHOGENICITY

The pathogenicity of the meningococcus has been described as irregular by Sophian (4). Flexner (5) found young guinea pigs of 125 grams to be the most susceptible animals. Elser and Huntcon (6) and von Lingelsheim and Leuchs (7) found that white mice succumbed most easily to injection of the meningococcus. In all animal tests the toxicity of the organisms (Sophian (4)) rather than their infective power has been regarded as the principal cause of death. A very important factor in the pathogenicity of the meningococcus is the age of the culture. Flexner (5) has shown that a culture is most viable when very young.

STANDARDIZATION OF SERUM

The bacteriotropic test as suggested by Jochmann (8) can not be conducted with uniform results.

Agglutination and complement fixation tests are most generally used at present but the tests are not made with standard reagents. The preparation of a standard antigen for the complement fixation test as recommended by Schwartz and McNeil (9) indicates a tendency toward uniformity in the test.

On account of the irregular pathogenicity of the meningococcus the animal protection test has not been used extensively. Flexner (5) found that his antimeningococcus serum would protect guinea pigs against one fatal dose of living organisms. Von Lingelsheim and Leuchs (7) could protect a white mouse against

one-half an agar slant with 0.5 cc. of immune serum. Amoss and Wollstein (10), consider a serum suitable for therapeutic use when it protects guinea pigs of 90 to 100 grams weight against a fatal infection.

EXPERIMENTAL

While testing the antimeningococcus serum produced at the Mulford Biological Laboratories, variations in the results of tests *in vitro* led us to believe that we were not recognizing the actual degree of immunity. The serum of one horse, 2059, after more than sixteen months of treatment would not give a complete fixation of complement in amounts of 0.01 cc. with meningococcus antigen. The serum of another horse, 2534, receiving similar treatment, gave complete fixation in amounts of 0.005 after three weeks.

In view of such discordant findings, the animal protection test was attempted. Since Flexner (5) has observed that salt solution is toxic for meningococci, fresh guinea pig serum diluted to four times its volume with salt solution (0.85 per cent), was used as a menstruum. A 16-hour old culture on serum-dextrose agar was uniformly employed. The organisms are thus suspended in a physiological medium and are in the most viable condition.

The technique of testing the virulence of a culture is as follows: the 16-hour growth is suspended in 1 cc. of dilute guinea pig serum; one half is immediately injected intraperitoneally into a white mouse; 0.5 cc. of the guinea pig serum is added to the remainder and one half of this suspension injected similarly into another mouse. Table 1 represents a typical protocol.

TABLE 1
Culture 40

WEIGHT OF MOUSE	AMOUNT OF AGAR SLANT	RESULT
<i>grams</i>		
17	0.5	Death in 18 hours
14	0.25	Death in 18 hours
13	0.12	Death in 40 hours
11	0.06	Death in 24 hours
11	0.03	Survived

In absolute point of time the mice do not die in regular order but at the end of forty-eight hours, after which no animals are affected, all receiving more than a certain amount of culture will be found dead. The cocci can be recovered invariably from the heart blood of the animals that succumb.

METHOD OF TESTING SERUM

When a serum is to be tested, it is injected in 0.5 cc. quantities, intraperitoneally two hours before the injection of the culture. The growth, on as many agar slants as there are tests to be made, is suspended in one cubic centimeter each, and pooled. By combining the growth from several agar slants each animal in a series receives exactly the same amount and the suspension is more uniform from time to time. The amounts of serum and bacterial suspension have been invariably 0.5 cc. each.

TABLE 2
Culture Para M

<i>Test March 29, 1916</i>	SERUMS					
AMOUNT OF AGAR SLANT	2059	2308	2526	2813	Normal	None
0.5	D. 24 hr.	D. 24 hr.	D. 20 hr.	D. 20 hr.	D. 20 hr.	D. 20 hr.
0.25	Lived	Lived	D. 20 hr.	D. 24 hr.	D. 40 hr.	D. 20 hr.
0.12	Lived	Lived	D. 48 hr.	D. 20 hr.	D. 24 hr.	D. 24 hr.
0.06	Lived	Lived	Lived	D. 24 hr.	D. 20 hr.	D. 48 hr.
0.03					D. 24 hr.	D. 24 hr.

Horse 2059 had been under immunizing treatment for sixteen months, 2308 for twelve months, 2526 for seven months, and 2813 for three months. Two weeks later the serum of both 2526 and 2813 protected against 0.12 agar slant per cubic centimeter.

These horses have been receiving injections of a polyvalent antigen according to the method of Amoss and Wollstein (10). Our collection of meningococci includes about fifty strains obtained from various sources, the majority being received through the kindness of Rockefeller Institute and the New York Board

of Health. It is our practice to combine these strains in equal parts in making the doses for the horses. It is manifestly impossible to test each bleeding against each strain. We have, however, been able to test one serum with all of the strains, and could demonstrate protection of greater or less degree against each one.

The tests with strains 62 (McWeeney) and L which Dr. Amoss of the Rockefeller Institute kindly informs us are strains of the normal meningococcus and parameningococcus, respectively, are given in tables 3 and 4.

TABLE 3
Culture 62 (McWeeney)

AMOUNT OF AGAR SLANT	SERUM 2050 A	RESULT	CONTROL ANIMALS NO SERUM
0.25	0.5	D. in 6 hours	D. in 18 hours
0.12	0.5	D. in 20 hours	D. in 18 hours
0.06	0.5	Survived	D. in 18 hours
0.03	0.5	Survived	Survived
0.015	0.5	Survived	Survived
0.007	0.5	Survived	Survived

TABLE 4
Culture Para L

AMOUNT OF AGAR SLANT	SERUM 2059 A	RESULT	CONTROL ANIMALS NO SERUM
0.25	0.5	D. in 18 hours	D. in 18 hours
0.12	0.5	Survived	D. in 18 hours
0.06	0.5	Survived	D. in 18 hours
0.03	0.5	Survived	D. in 18 hours
0.015	0.5	Survived	D. in 20 hours
0.007	0.5	Survived	D. in 20 hours

SPECIFICITY OF THE TEST

There seems to be a considerable degree of specificity in the test. The serum of horse 2629, after treatment for four months with Strain 1 (Isadore) gave the results shown in tables 5 and 6.

TABLE 5

Strain 1

AMOUNT OF AGAR SLANT	SERUM 2629	RESULT	SERUM	RESULT
0.5	0.5	D. 40 hours	0	D. in 24 hours
0.25	0.5	D. 24 hours	0	D. in 20 hours
0.12	0.5	Survived	0	D. in 24 hours
0.06	0.5	Survived	0	D. in 20 hours

TABLE 6

Strain Para M

AMOUNT OF AGAR SLANT	SERUM 2629	RESULT	SERUM	RESULT	GONO- COCCUS GOAT SERUM	RESULT
0.5	0.5	D. 40 hours	0	D. 20 hours	0.5	D. 20 hours
0.25	0.5	D. 20 hours	0	D. 20 hours	0.5	D. 20 hours
0.12	0.5	D. 20 hours	0	D. 20 hours	0.5	D. 20 hours
0.06	0.5	D. 20 hours	0	D. 20 hours	0.5	D. 20 hours

CLASSIFICATION OF MENINGOCOCCI

Classification of the meningococci by this test has not as yet progressed far enough to permit a report. The results of this work will undoubtedly allow us to make a more nearly balanced treatment of the horses in regard to groups.

INCREASE OF VIRULENCE

Increasing the virulence of a meningococcus strain by animal passage is also under consideration at present.

CORRELATION OF OTHER SEROLOGICAL REACTIONS

We have to some extent, tried to correlate the results of agglutination and complement fixation tests with the protective power of the serum (table 7). Agglutination tests were made with the same strain as was used in the protective test. Fresh serum was used and the results were read after twenty-four hours at 55°C. The antigen employed for complement fixation was a fresh suspension of equal parts of all strains used in immunizing the horses. As a rule a high agglutinating titer is accom-

panied by strong complement fixing power but does not imply ability to protect against an infection.

TABLE 7

SERUM	PROTECTIVE UNITS PER CC.,* PARA M	FIXATION OF COMPLEMENT; MIXED ANTIGEN	AGGLUTINATION PARA M
2059T	16	0.01 +++	1:80
2308H	4	0.002 ++++	1:80
2334K	8	0.001 ++++	1:160
2524N	4	0.001 ++++	1:160
2525D	8	0.002 ++++	1:40
2526K	4	0.01 ++++	1:80
2627B†	0	0.002 ++++	1:40

* Owing to the fact that the controls were not carried to the M.L.D. the protective units in this table are comparative and not actual.

† This horse was immunized against Strain 1.

The final test of the efficiency of a therapeutic serum is the result of its use in human infection. We have obtained two cultures of meningococci from cases in which our serum was used. Both cases showed considerable improvement after the injection of the serum and eventually recovered completely. The results of the tests as to the protective power of the serum against infection with these two strains are shown in table 8.

TABLE 8
Culture 1569

AMOUNT OF AGAR SLANT	SERUM 2059 V	RESULT	CONTROL ANIMALS NO SERUM
0.5	0.5	Survived	Death in 18 hours
0.25	0.5	Survived	Death in 18 hours
0.12	0.5	Survived	Death in 24 hours
0.06	0.5	Survived	Survived
0.03			Survived
0.015			Survived

Culture 1594

0.5	0.5	D. 18 hours	Death in 18 hours
0.25	0.5	D. 40 hours	Death in 24 hours
0.12	0.5	Survived	Death in 18 hours
0.06	0.5	Survived	Death in 18 hours
0.03			Death in 18 hours
0.015			Survived

DISCUSSION

To the average practitioner, a standardization according to protective units means more than a standardization that is based on the complement fixing or agglutinating titer of a serum. The protective power of a serum is undoubtedly a better index of therapeutic value than any test *in vitro*. The test described above is suggested as the basis for a uniform method of standardizing antimeningococcus serum. The logical unit of value would be the amount of serum necessary to protect against one fatal dose of culture.

It is not possible at the present time, considering our incomplete knowledge of the relationship of different strains, to recommend standard strains for such tests. If all strains are equally potent in stimulating the formation of protective antibodies, a strain from each group, selected at random, should measure the value of the serum. Since the amount of protection that a serum gives will depend considerably upon the virulence of the culture used, it may be advisable to stipulate that the culture should kill a mouse in amounts of 0.01 or 0.001 of an agar slant. Thus, a serum which would protect against 0.1 agar slant of a culture the M.L.D. of which was 0.001 agar slant, could be said to contain 100 units per cubic centimeter.

From the standpoint of the practitioner it is desirable that a standard dose of antimeningococcus serum should contain perhaps 1500 units. This would resemble, in number of units, a dose of antitoxic serum. A serum protecting against 100 M.L.D. per cubic centimeter given in 15 cc. doses would contain 1500 units. If, on the other hand, the test culture was of comparatively low virulence, the serum might show only 10 units per cubic centimeter. If it becomes necessary to use such a culture, we could assume, in view of the method of standardizing the antitoxic sera, a unit to be one-tenth the amount of serum necessary to protect against a fatal dose of culture. The final decision as to a uniform method must await an extensive study of the meningococcus cultures.

Our tests have demonstrated the great importance of a poly-

valent serum. Until we have grouped the meningococci around type strains, it is our policy to test each newly isolated culture against a polyvalent serum. Protection can be demonstrated against the majority of new strains. Those against which the serum shows no protection are immediately incorporated into the treatment for the horses. For the routine testing of the serum, certain comparatively virulent strains are used.

SUMMARY

By suspending a 16-hour meningococcus culture in sterile fresh guinea pig's serum, diluted to four times its volume, and injecting this suspension intraperitoneally into white mice, a regular virulence can be demonstrated.

If the amount of serum necessary to protect against one M.L.D. of culture is considered as a unit, a rational and uniform method of standardizing antimeningococcus serum can be obtained.

A dosage similar to that of antitoxic serum would be possible by such a method of standardization.

The protection test parallels the extent of immunization more nearly than agglutination or complement fixation tests.

The protection test is specific, differentiating strains of meningococci as well as the gonococcus.

The polyvalency of an antimeningococcus serum is of extreme importance.

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STUDIES ON ANTIBACTERIAL SERUMS

II. A STABLE BACTERIAL ANTIGEN: WITH SPECIAL REFERENCE TO MENINGOCOCCI

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Among the several methods suggested for the titration of anti-meningococcus serum, complement fixation was first recommended by Kolle and Wassermann (1). They estimated that a serum to be valuable for treatment should inhibit hemolysis in a dose of 0.001 cc. Other writers, however, chiefly Krumbein and Diehl (2) and St. Baecher and Hachla (3) claimed that this method of testing may lead to erroneous results. Indeed, according to Neufeld (4) and Neufeld and Händel (5) the complement fixing antibodies are quite independent of the bacteriolytic amboceptors and do not necessarily bear any relation to the therapeutic value of the serum.

A similar view has been held by American investigators. Titration of the bacteriotropic power of the serum according to the method of Neufeld as modified by Jobling and Lamar was accepted by Flexner. The laboratories of this country adopted the bacteriotropic method as the most rational of the tests since it was believed to imitate the mode of action of the serum therapeutically.

Recently, however, there has been a reaction against this method because of the well-recognized inaccuracies of the test. The slightest differences in technique, even those attributable to personal equation, were the occasion of such wide differences in result that the standard in one laboratory could be hardly comparable to that in another.

The need of a method capable of producing uniform results, therefore, brought about a return to the complement fixation test. It was, of course, realized that the objection made by Neufeld was probably well founded. But on the other hand, serum with high complement-fixing power for the various types of *meningococcus* would indicate that the animal, from which the serum in question was obtained, had been sufficiently immunized to produce the other antibodies necessary for therapeutic activity. A serum considered to be sufficiently potent for purposes of treatment showed complement fixing power in a quantity of 0.002 cc.

An important problem in the complement fixation test is the preparation of the antigen.

The various methods in common use for this purpose may be divided into three classes:

1. Bacterial suspension: (a) Fresh (unchanged); (b) Stock suspension which naturally must contain varying amounts of autolyzed bacteria and extractive matters.

2. Autolyzed bacteria.

3. Filtrates.

An emulsion of meningococci must be prepared *ex tempore* and used when fresh, if one is to consider the unchanged proteins of the bacteria a part of the necessary constituents. It is self-evident, then, that by attempting to use fresh bacterial suspensions we introduce many possible sources of variation in the result.

Autolysates and filtrates, on the other hand, may be prepared in quantity and stored for a considerable period. They are, however, subject to sudden changes in titer; even though portions of the same lot may be distributed to various laboratories, the slightly different conditions under which they are kept may lead to considerable differences in the combining power of the antigens. Possible bacterial contamination is another disadvantage.

If we can prepare an antigen that is not subject to alteration or at least to only very slight alteration due to storage or changes in temperature, we shall have eliminated one of the possible sources of difference in the results of complement fixation titration of antimeningococcus serum.

This end has been accomplished, we believe, by the preparation of a dried meningococcus antigen.

If prepared in large quantities and stored *in vacuo* with phosphorus pentoxid this antigen will remain stable for a long period. Under these conditions an antigen prepared in one laboratory could be distributed to other laboratories with the assurance that the antigen element in the complement fixation technique would be uniform.

Furthermore, an antigen prepared in this way would have the advantage of being specific for a single type of meningococcus or polyvalent in definite proportions for all the several types of the organism. It is well known that certain strains autolyze more rapidly than others and by the method suggested the influence of this factor is minimized.

The technique for preparing the dried meningococcus antigen is as follows:

1. The cultures are grown on salt-free agar at 37°C. for sixteen to eighteen hours.
2. The growth is collected and suspended in distilled water; about 10 cc. distilled water being used for each 20 square inches of agar surface.
3. To this suspension is added an equal volume of 95 per cent ethyl alcohol.
4. This mixture is immediately centrifugalized.
5. After the supernatant fluid has been removed the precipitated bacteria are again suspended in 95 per cent ethyl alcohol.
6. This process of centrifugalization and resuspension is repeated a second and a third time, but with ethyl ether instead of alcohol, the original volume of the suspension being reduced to one-half each time.
7. The ether adhering to the bacteria after the last precipitation and after removal of the supernatant ether, is removed by vacuum.
8. The bacterial mass now constituting the antigen is further dried over phosphorus pentoxid *in vacuo* for three days.
9. The antigen is stored in tubes—about 0.05 gram to each—and kept *in vacuo* over phosphorus pentoxid.

For storing the antigen under these conditions we have used the well-known "H" tube. This is made by joining two test tubes, 1 inch in diameter by 4 inches long, at about their middle third with a narrower tube thus forming the letter "H."

The antigen is placed in one tube; the P_2O_5 in the other. In order to prevent mixing of the contents, the connecting tube is loosely plugged with cotton. The open end of the " P_2O_5 " tube is then sealed, the other is drawn down to capillary size and connected with the vacuum pump. After exhaustion of the air, this tube also is sealed in the blow-pipe flame.

The antigen is stored in the dark in a refrigerator.

Suspensions for use are prepared as follows:

A small amount of the powder, say 0.02 gram is carefully ground in a mortar, 20 cc. of physiological saline solution being gradually added. We thus obtain a suspension 1:1000 based upon the dry weight of the antigen. The anticomplementary action of this suspension is determined in the usual manner. One cubic centimeter of a suspension, 1:10,000, usually shows slight inhibitive power, and 1 cc. of a suspension, 1:50,000, still has sufficient combining power to anchor all the complement contained in 1 cc. of a dilution, 1:20, if a sufficient amount of specific serum is present.

This antigen is therefore used in dilutions 1:20,000 to 1:30,000, a sufficient combining dose being thus obtained and at the same time a suspension of such high dilution that its anticomplementary properties do not interfere with the specific results. To guard against error, however, it is advisable always to set up control tubes containing a double amount of the antigen and the hemolytic system in parallel series with the actual test.

Below are appended protocols showing the specificity of the antigen.

TABLE 1

VARIOUS ANTIGENS	AMOUNT OF ANTIMENINGOCOCCUS SERUM IN CUBIC CENTIMETERS								
	0.05	0.04	0.03	0.02	0.01	0.008	0.006	0.004	0.002
Suspension of dried bacteria.	++++	++++	++++	++++	++++	+++	+++	++	++
Suspension of fresh cultures.	++++	++++	++++	++++	++++	+++	+++	++	++
Autolysate, McNeal	++++	+++	+++	+++	0	0	0	0	0
Cholesterin, fortified	+	+	+	0	0	0	0	0	0
Noguchi	0	0	0	0	0	0	0	0	0
Gonococcus	+	+	0	0	0	0	0	0	0
Catarrhalis	0	0	0	0	0	0	0	0	0
Staphylococcus..	0	0	0	0	0	0	0	0	0
Pneumococcus (polyvalent) ..	+	0	0	0	0	0	0	0	0
None (control) ..	0	0	0	0	0	0	0	0	0

In this series of tests the antimeningococcus serum is the constant; the amounts of serum used are shown at the top of the columns. The antigen in each case is different. The very slight inhibition of hemolysis shown in certain instances with the heterologous antigens is negligible.

TABLE 2

Test with various serums and the dried meningococci antigen

		AMOUNT OF SERUM EXPRESSED IN CUBIC CENTIMETERS								
		0.05	0.04	0.03	0.02	0.01	0.008	0.006	0.004	0.002
Antimeningitis heated to 56°C. ½ hour	Tricresol 0.4 per cent.	0	0	0	0	0	0	0	0	0
	Control.	0	0	0	0	0	0	0	0	0
	No preservative.	+++	++	++	++	0	0	0	0	0
	Control.	0	0	0	0	0	0	0	0	0
	Tricresol 0.4 per cent.	++++	++++	++++	++++	++++	++++	+++	++	+
	Control.	+++	++	++	+	0	0	0	0	0
Antimeningitis unheated	No preservative.	++++	++++	++++	++++	+++	++	++	++	+
	Control.	+	+	+	0	0	0	0	0	0
	Test.	0	0	0	0	0	0	0	0	0
	Control.	0	0	0	0	0	0	0	0	0
	Test.	0	0	0	0	0	0	0	0	0
	Control.	0	0	0	0	0	0	0	0	0
Anti Cat.	Test.	0	0	0	0	0	0	0	0	0
	Control.	0	0	0	0	0	0	0	0	0
Anti Gon.	Test.	0	0	0	0	0	0	0	0	0
	Control.	0	0	0	0	0	0	0	0	0
Anti Pneumo.	Test.	+	0	0	0	0	0	0	0	0
	Control.	+	0	0	0	0	0	0	0	0
Anti Staph.	Test.	0	0	0	0	0	0	0	0	0
	Control.	0	0	0	0	0	0	0	0	0
Anti Strep.	Test.	0	0	0	0	0	0	0	0	0
	Control.	0	0	0	0	0	0	0	0	0

In this table the dried meningococcus antigen is the constant. In the column at the left are shown the various serums used. It is to be noted that tricresolized antimeningococcus serum heated to 56°C. for ½ hour has lost its complement binding power in the dilutions used. Heat alone greatly reduces the complement binding power even without tricresol.

TABLE 3

The control serums and their respective antigens

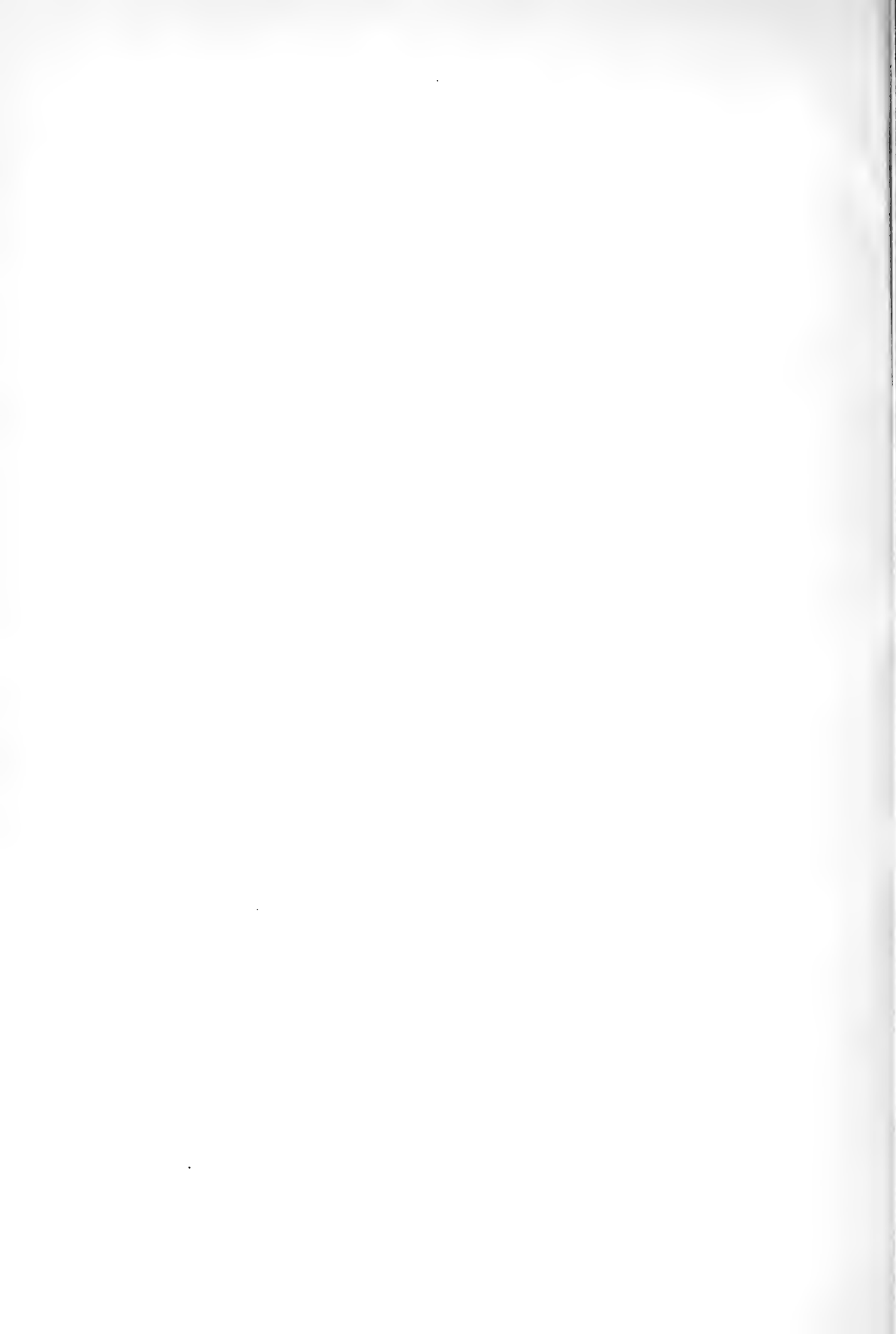
	AMOUNT OF SERUM								
	0.05	0.04	0.03	0.02	0.01	0.008	0.006	0.004	0.002
Catarrhalis.....	++++	++++	+++	++	0	0	0	0	0
Control.....	+	0	0	0	0	0	0	0	0
Streptococcus....	+++	+++	+++	++	0	0	0	0	0
Control.....	0	0	0	0	0	0	0	0	0
Pneumococcus....	++++	++++	+++	++	+	0	0	0	0
Control.....	+	+		0	0	0	0	0	0
Gonococcus.....	+++	+++	++	+	0	0	0	0	0
Control.....	0	0	0	0	0	0	0	0	0
Staphylococcus..	++++	++++	+++	++	+	0	0	0	0
Control.....	+	+	0	0	0	0	0	0	0

This table shows that the serums used to control the meningococcus antigen possessed specific activity for their homologous antigens; it also shows that the control antigens were active.

Up to the present our technique has been applied only to the meningococcus. It is hoped, however, that experiments now under way will demonstrate the possibility of preparing a dried and stable gonococcus antigen, the value of which would be obvious; a gonococcus complement fixation test properly carried out is capable of giving the clinician valuable information, but largely owing to the worthlessness of the antigens too commonly used, we believe the gonococcus complement fixation test has not received the attention it deserves.

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ELECTIVE LOCALIZATION OF THE STREPTOCOCCUS FROM A CASE OF PULPITIS, DENTAL NEURITIS AND MYOSITIS¹

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Localized infections in or about the roots of teeth have already been considered in causal relationship to systemic disease and to neuralgia of the face. Experimental proof of the nature of this relationship, however, is still undetermined. In this paper I wish to record the history of a case in point and the results of experiments which appear to throw light on the subject.

Case 567. Mrs. F. H. P., aged thirty-five years, had been subject to severe migraine for many years. For the past five or six years she has suffered from recurring attacks of neuralgia of the face, which began with severe pain and distinct swelling over the left upper jaw, spreading to the opposite side, with soreness in the teeth, especially in the upper jaw, and followed by intense pain in the left side of the head, neck and shoulders. During the last two or three years the attacks have occurred oftener and had grown so severe as to necessitate the frequent use of hypodermics of morphine; the attacks have ended with spasm of the muscles, and tenderness and swelling of the neck on the left side. Her tonsils were removed four years ago, but this did not relieve the condition. One year ago the second left upper molar showing a blind abscess at the root was extracted, the left maxillary sinus drained and a piece of the left turbinate removed, but without relief. She became extremely nervous, at times hysterical during the paroxysmal pain, and has had one or two spells of mental confusion suggesting *petit mal*. Previous to tonsillectomy, she had had for years one or more attacks of tonsillitis followed by rheumatic pains during the winter months.

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Examination revealed a poor vasomotor tone, moderately firm muscles, fair nutrition, and the general appearance of a nervous woman. On January 13, the hemoglobin was 85 per cent; the leukocytes 9800. A Wassermann test of the blood proved negative. On February 17, the hemoglobin was 80 per cent; the erythrocytes 4,210,000; the leukocytes 5400. There were no signs of organic disease of the central nervous system. The examination of the heart, lungs, abdomen, reflexes, urine, Roentgen examination of the jaws, roots of teeth, and the blood pressure, were all negative. There was tenderness over the left infra-orbital foramen and mental foramen, but no superficial tenderness of the overlying skin. There were tender nodules, which appeared to be enlarged glands in the posterior triangle of the left side of the neck. There was a clean tonsillectomy scar, a normal condition of the nose except moderate hypertrophic rhinitis, and a normal condition of the gums and teeth, except a tender dead first upper left molar which had been crowned, but from which the crown had been removed on account of the irritation of the gums. The maxillary sinuses were clear. Owing to the fact that each attack began with swelling of the left upper jaw opposite the dead tooth and in the region of the left infraorbital foramen, the tooth was extracted January 18. There was found a semi-lunar eroded area 2 mm. in diameter near the apex of the largest and inner fang. The surface of this was sterilized with a searing blade, and the root-canal drilled into from the apex with a dental burr. The pulp-cavity was filled with a foul-smelling pus. The erosion was situated so that it was impossible to be shown by the Roentgen ray. The pulp-cavity of the other two fangs was filled and obliterated and there were no erosions at the apices. There was a large cement filling directly opposite the dead pulp. Smears from the pus from the root showed a few Gram positive diplococci, diptheroid bacilli and a few Gram negative bacilli. The primary cultures in ascites-dextrose agar and broth gave a pure culture of a slightly hemolyzing streptococcus. The anerobic cultures on blood agar slants and in tall columns of ascites fluid containing sterile tissue, covered with paraffin oil, had a foul odor and many short chains of streptococci and numerous bacilli resembling *Bacillus fusiformis*.

Immediately after the extraction of the tooth, the patient developed an unusually severe attack but she gradually recovered after several exacerbations. On February 17, after having been free from pain for ten days and gaining rapidly in weight and strength, there was found a definite tenderness of the muscles in the posterior triangle on the left

side of the neck and two distinct tender nodules just behind the posterior margin of the sternomastoid muscle. One of these tender nodules, thought to be a lymph-gland, and a portion of the deeper layers of the muscle, was excised. One-half of these tissues were immediately emulsified for cultures and the other half fixed in formalin-Zenker for sections. The excision of the fascia and muscle precipitated another violent attack of pain and spasm of the muscles of the left side of the neck. The cultures in tall columns of ascites plain broth of the emulsion of muscle showed a pure culture of a short chained streptococcus and those from the thickened fascia, streptococci and staphylococci.

Blood agar plates from the culture in ascites plain broth from the tooth pulp and from the muscle (injected into animals) showed pure cultures of streptococci producing a narrow hazy zone of hemolysis. It was thought that this streptococcus might be present quite generally on the mucous membrane in this patient. Cultures from the nose and pharynx and three out of six cultures from the stool proved that this was actually the case.

Sections of the excised muscle showed marked increase in the interstitial tissue, poorly staining nuclei of adjacent muscle fibers, and slight round cell infiltration (Fig. 1). Sections of the fibrous nodule and fascia showed old and young connective tissue, absence of lymphoid tissue, small nests of round cells, plasma cells, and erythrocytes, chiefly around blood vessels. Gram-Weigert and methylene blue stains for bacteria revealed a moderate number of diplococci in or adjacent to the fibrous tissue between the muscle fibers and nests of cellular infiltration (Fig. 2) and a large number within and surrounding a small sized blood vessel in the center of the fibrous nodule (Fig. 3).

A vaccine was prepared by heating the streptococci suspended in salt solution from the primary cultures of the pulp of the tooth and muscle and treating it with equal parts of the patient's serum for two hours at 37°F and over night in the ice chest. This was used in the treatment of the patient. The first dose consisted of 25,000,000, and was followed by marked muscle pains, especially at the left side of the neck, by nausea, extreme exhaustion and slight fever. The subsequent injections were at first diminished and then gradually increased, aiming to give a distinct but not severe reaction following the injection. Dr. Grimes, who referred the patient to me, reported three months after the extraction of the tooth, that the patient has shown gradual improvement; the attacks are milder and of shorter duration, the intervals between attacks are longer, and there has been marked improvement in the general nervous tone.

ANIMAL EXPERIMENTS

Intravenous injections were made into rabbits, guinea-pigs and dogs, and intraperitoneal injections into mice. The streptococcus as isolated from the pulp of the tooth was injected into 4 rabbits, 2 guinea-pigs and 2 white mice, all of which recovered. The primary culture after one animal passage was injected into 2 rabbits, 1 dog and 2 mice. The rabbits and dog recovered; the mice died. Thirteen animals were injected. In 10 of these circumscribed hemorrhages and edema opposite the roots of teeth or at the foramina of exit of the superior or inferior maxillary nerves were easily visible; in 7 there were lesions of the muscles, 4 of which were limited largely to the muscles of the left side of the neck; in 6 there were lesions of the pulp of the teeth and the superior or inferior dental nerves; and in 3 there were insignificant lesions of the kidney, 2 in the gall bladder and 1 in the stomach. The spleen, adrenal, endocardium, vagus and sympathetic ganglia and subcutaneous nerves were normal. No lesions were found in the nerves supplying hemorrhagic muscles. The diptheroid bacillus did not produce lesions in the one rabbit and one mouse injected.

The streptococcus from the muscle in the first and second cultures was injected into 2 rabbits, 1 guinea-pig and 1 mouse, producing lesions of the muscles, chiefly of the neck and shoulders, in all. In the rabbits there were also hemorrhage and infiltration of the dental nerves, and gross lesions in the dental pulps and periosteum opposite the roots of the teeth. In 1 there were a few hemorrhages of the stomach, in the other, hemorrhages of the kidney; in the mice there was a mild peritonitis.

To the portion of the above cultures of streptococci left over and having marked affinity for the muscles, was added 0.5 per cent formalin. This was allowed to stand over night at room temperature, a portion put aside for direct injection; the rest was centrifuged and the bacteria washed in Ringer's solution. The former was injected intravenously into a rabbit in amounts of 10 cc. on four occasions over a period of eleven days. The rabbit showed a moderate number of small hemorrhages in the

muscles of the hips and shoulders and a few in the stomach. The washed suspension was injected one month later into 3 rabbits, each receiving the growth from 150 cc. of the broth cultures. All died in twenty-four hours. The muscles in all had a boiled appearance, and those about the shoulders, neck and spine had large and small hemorrhages associated with edema; 2 had, in addition, a hemorrhage of the pulp of the teeth; and 1 a few hemorrhages about the joints, in the tricuspid valve and in the left inferior dental nerve. The lungs were free from hemorrhages.

Two cubic centimeters or approximately 500,000,000 of the heat-killed streptococci failed to produce lesions, but this may have been due to the small size of the dose. The filtrate of the formalinized cultures failed to produce lesions in the muscles following 3 injections of 10 cc. each into 1 rabbit. Three injections into 1 rabbit over a period of eleven days of the slightly hemolyzing streptococcus from the pharynx were followed by moderate numbers of lesions in the muscles in the right side of the neck, the right elbow and right shoulder and in the intercostal muscles. The streptococcus from the stools in the second culture did not produce lesions. The living streptococci from the tooth, muscle, pharynx and stool in the first or second cultures, and after one animal passage, and the heat or formalin-killed streptococci, were injected altogether into 24 animals (15 rabbits, 3 guinea pigs, 1 dog and 5 mice). Of these 17 (71 per cent) showed myositis, 13 (54 per cent) lesions of the periosteum opposite teeth or at nerve foramina, 12 (50 per cent) lesions of the pulp of teeth and 10 (42 per cent) lesions of the dental nerves.² Similar results have been obtained since in 2 cases of myositis and arthritis with streptococci isolated from the diseased pulp of extracted teeth.

Previous to these experiments lesions of the pulp of teeth and the dental nerves were not usually looked for, but recently careful search for similar lesions in these structures has been made

² The incidence of the lesions in the other organs corresponded with those given in my paper on "Elective localization of streptococci," Jour. A. M. A., 1915, 45, 1687-1691.

in numerous animals injected with streptococci from other sources. In three instances only were there found lesions of the pulp of teeth and dental nerves. Two of these animals showed myositis in addition. The details in the following experiments will serve to illustrate specifically the results obtained:

R 656. Belgian hare, 1510 grams weight. January 19, 1916, injected intravenously with the growth from 30 cc. of ascites-dextrose-broth of a pure culture of streptococcus from the pulp of the extracted tooth.

January 20. Seemed quite ill; respirations were accelerated; appeared to have pain in walking and promptly crouched when quiet. The hair was roughened and there was marked lacrimation of the left eye, but no swelling of the face.

January 21. Seemed much better; was more active but tremulous, and appeared nervous. There was lacrimation of the left eye and an easily recognizable swelling of the left side of the face (Fig. 4). Slight pressure over this area appeared to cause pain and the swelling could be easily felt. Chloroformed. A rather large number of linear hemorrhages in the skeletal muscles, chiefly in the tendonous portions of the flat muscles of the shoulder and deeper muscles of the left side of the neck and of the front extremities, were found. There were no lesions of the muscles of the hind extremities, the dorsal and lumbar regions of the spine, nor of the intercostal muscles and diaphragm. On removing the skin on the left side of the face, marked edema, infiltration and hemorrhage of the subcutaneous tissue, the fascia, the muscles and the periosteum, were found. The hemorrhages in the periosteum opposite the molars appeared to be the center of the edematous area. The hemorrhagic infiltration extended to the under surface of the orbit. The pulp of the left third and fourth upper molars was found to be edematous and hemorrhagic. The hemorrhages were small and punctate and were not found in the pulp of the 2 corresponding teeth on the opposite side. On dissecting away the soft tissues of the lower jaw, it was found that hemorrhages of the periosteum had occurred opposite the apices of the right lower incisor, and the first and two last molars, the second left lower molar, and surrounding the right mental foramen similarly to the lesions shown in Fig. 6. The pulp of the teeth surrounded by hemorrhages in the periosteum was found to be edematous and hemorrhagic, whereas 3 adjacent pulps appeared normal. The left superior dental nerve was extremely hyperemic and a number

of small hemorrhages were found in the sheath. The only lesions of the viscera were 3 small hemorrhages in the mucous membrane of the pyloric end of the stomach and 3 small subserous hemorrhages at the fundus of the gallbladder.

January 23. Blood-agar plate cultures of the blood produced 1 colony of streptococcus; of the emulsions of the pulp of teeth 5 and 18 colonies respectively. Cultures in ascites-dextrose broth from the emulsions of the pulp of 2 teeth, of the hemorrhagic muscle, of the edematous tissue over the jaw and of the hemorrhagic periosteum, showed a short chained streptococcus.

Sections of the edematous tissue over the left jaw showed extreme hemorrhage, edema and beginning leucocytic infiltration. In the pulp of 2 teeth from which sections were made, were large and small hemorrhages chiefly beneath the layer of odontoblasts, and in the left superior dental nerve were small areas of hemorrhage in the sheath. Sections of hemorrhagic areas in the scapular muscles showed marked hemorrhage between the muscle fibers, separation and fragmentation of the latter, and slight leukocytic infiltration. Gram-Weigert stains showed diplococci in or adjacent to the hemorrhagic areas in the edematous periosteum, in the muscle from the scapula, in the pulp of the teeth, and in the left superior dental nerve (Fig. 5).

D 412. A white and black dog, weight 9.5 kilos. January 22, injected intravenously with the growth from 90 cc. of ascites-dextrose broth of the streptococcus from the hemorrhagic pulp of a tooth of the above rabbit.

January 24. Seemed well; was active; no swelling of the face; no tenderness over joints nor muscles. Chloroformed. The left inferior dental nerve was found to be edematous and hyperemic and contained a number of large and small punctate hemorrhages (Fig. 7). The pulp of the corresponding canine tooth likewise appeared edematous and contained numerous punctate hemorrhages. The corresponding nerve and pulp on the opposite side appeared normal (Fig. 7). There were similar but less marked lesions of the left superior dental nerve and pulp of the canine tooth. The first upper and lower molars were examined and only the first left lower molar showed unmistakable hemorrhage. The muscles were free from visible lesions except for a few hemorrhages in the flat muscles under the scapulae. In a painstaking examination of all the organs no other apparent lesions were revealed.

January 25. Blood agar slants made from the blood were sterile.

Sections for microscopic study were made from the left inferior dental nerve, from the pulp of the teeth mentioned, and from the superior maxillary nerve. Hemorrhages and beginning leucocytic infiltration were found in the pulp of the left inferior canine (Fig. 8) and a small number of hemorrhages in the pulp of the first left lower molar, but practically none in the others. These hemorrhages were most numerous immediately beneath the layer of odontoblasts and at the distal portion of the pulp (Fig. 8). Sections of the left inferior dental nerve showed marked edema, a moderate leucocytic infiltration and a number of large and small hemorrhages chiefly in the sheath (Fig. 10). There were no lesions in the left superior maxillary nerve. Gram-Weigert and methylene blue stains for bacteria showed scattered diplococci and at times chains of diplococci in and adjoining the hemorrhagic areas (Figs. 9 and 11). In one instance, a mass of diplococci surrounded by erythrocytes was found just outside the wall, and 2 diplococci in the wall of a small blood vessel. In a number of instances the diplococci were found within leucocytes and in what appeared to be endothelial cells. No bacteria could be found in the normal portions of the pulps and nerves showing lesions, nor in those free from lesions.

P 155. White guinea-pig, weight 320 grams. February 19, injected intravenously with the growth from 30 cc. of ascites-dextrose broth of the streptococcus isolated from the muscle of the patient.

February 21. Seemed quite well, but appeared to be muscle-sore. Chloroformed. Numerous small hemorrhages associated with edema in the triceps muscles and a moderate number in the muscles of the left shoulder and a few hemorrhages in the deeper layer of the muscles of the left side of the neck were found. There were no lesions of the dental nerves but the pulp of the two left upper molars was extremely hyperemic and contained distinct hemorrhages. There were no other findable lesions.

February 24. Cultures from the blood in ascites-dextrose broth were sterile.

Sections through the areas of hemorrhage in the scapular muscle showed marked extravasation of blood corpuscles, leucocytic infiltration, separation and necrosis of muscle fibers (see Fig. 14 illustrating similar lesion in triceps muscle). Sections of the pulp of the left first upper molar showed a number of small hemorrhages while in those of the superior maxillary and right superior dental nerve there were no lesions. Gram-Weigert and methylene blue and eosin stains showed a few scattered diplococci in the hemorrhagic and infiltrated area in the muscle and adjacent to the hemorrhages in the tooth-pulp (Fig. 15).

THE STREPTOCOCCUS

The streptococcus isolated from the dead pulp of the tooth, the muscle, the pharynx, and the stool, produced small, round, slightly elevated grayish-brown, non-adherent, rather dry, colonies on aerobic blood (human) agar plates. In broth it produced a diffuse turbidity with a small amount of flocculent sediment at the end of forty-eight hours. It acidified and coagulated milk. In liquid media it grew in short chains and diplococci. In many instances, the single members of the diplococci were quite round and resembled staphylococci. The strains from the tooth and muscle were freely susceptible to phagocytosis and the virulence was of a low order. The streptococci from blood agar slants in salt solution used as a vaccine resisted 60°C. for thirty minutes on consecutive days, while after the third heating the subcultures remained sterile. The blood of the animals soon became sterile, and nearly all recovered promptly from the effects of injection. The organism resembled very closely the streptococci that I have isolated from other cases of myositis and strains from other sources having affinity for muscles.

In Table 1 is given the fermentative power of the strains isolated from the pulp of the tooth, the muscle, the pharynx, and the stool. It is seen here, as has been found to be the case with

TABLE 1
*Fermentative powers of the streptococcus**

STREPTOCOCCUS	DEXTROSE	LACTOSE	SACCHAROSE	MALTOSE	RAFFINOSE	MANNITE	SALICIN	INULIN
Tooth pulp.....	2.4	1.5	0.9	1.9	0	1.3	2.0	0
Pharynx.....	1.9	1.8	0	1.6	1.8	1.0	0	0
Muscle.....	1.5	1.2	1.5	1.3	0	0	0	0
Stool.....	2.9	2.3	1.1	3.2	0	0	2.3	0

* The standard sugar-free broth containing the usual amounts of the various carbohydrates was used in the fermentative tests. The cultures were incubated 72 hours. The figures indicate the number of cubic centimeters of normal tenth sodium hydrate required to neutralize 5 cc. of the broth culture, phenolphthalein being used as an indicator.

other streptococci, that there is little parallelism between pathogenicity and the degree or range of fermentative powers.

SUMMARY

A streptococcus having peculiar properties was isolated from the dead pulp of the left upper first molar in the region where the attacks of pain usually began. The streptococcus was also demonstrated in the sections and isolated from the infiltrated deep fascia and muscles of the left side of the neck. A similar streptococcus was isolated from the pharynx and stool. This streptococcus was proved to have elective affinity for the pulp of teeth, dental nerves and muscles in animals. It was repeatedly isolated from and demonstrated in the experimental lesions in animals whose blood was sterile; the lesions were again produced on reinjection and the streptococcus again isolated. Many animals appeared to be in pain and 1 rabbit (Fig. 4) had marked swelling and tenderness over the left upper jaw. This affinity was proved absent in the diphtheroid bacillus and *Bacillus fusiformis*, which also were isolated from the pulp of the tooth, and in the streptococcus broth culture filtrate. Streptococci from other sources rarely cause lesions in the pulp of teeth and dental nerves. The phagocytic power of the patient's blood following the attack over the strain from the tooth was twice that of comparable normal blood.

These results would appear to warrant drawing the conclusion that the attacks of pain in the face in this patient were due to a streptococcus infection of the sheaths of the dental nerves, and that the pain, swelling, tenderness and spasm of the muscles of the neck were due to myositis and fibrositis—the result of infection by this streptococcus. The demonstration of living streptococci in the pulp of the tooth and in the fascia and muscle during quiescent intervals is significant and may explain the recurrence of the attacks. The cavity in the tooth containing the dead pulp, which was originally infected from the mouth, judging by the character of the filling and of the bacterial flora, was quite unable to heal for mechanical reasons. The contents

of the cavity appeared to afford a culture medium for the growth of the streptococcus. From stimulation of the defensive mechanism in the patient during the attacks, active growth appeared to be held in check and the symptoms disappeared in consequence, only to reappear later from recurrence of active growth and localization of the streptococci when the immunity was low.

The improvement in the patient since extraction of the tooth appears to be due to the removal of this focus and to prolonged artificial stimulation of the defensive mechanism by means of the autogenous vaccine, which, it is hoped, will lead to the destruction of all the streptococci in the muscle and dental nerves, and result in the ultimate recovery of the patient. However, the isolation of this streptococcus from so many places, indicates that complete recovery will probably be difficult.

PLATE 1

FIG. 2. Diplococci, singly and in short chains, and two leucocytes in tender fibrous nodule in the deep fascia from the left side of the neck. Methylene blue and eosin. $\times 1000$.

FIG. 3. Diplococci within the lumen and in the wall of the blood vessel and within the leucocyte just outside of the blood vessel in the center of the tender fibrous nodule of the deep fascia from the left side of the neck. Gram-Weigert. $\times 1000$.

FIG. 5. Diplococcus in an area of hemorrhage in the left superior dental nerve of rabbit shown in Fig. 4. Gram-Weigert. $\times 1000$.

FIG. 9. Diplococci, singly and in short chains, within and outside leucocytes, adjacent to the area of hemorrhage shown at *a* in Fig. 8.

FIG. 11. Diplococci and double chain of streptococci in the hemorrhagic and infiltrated area (*a*) in the sheath, shown in Fig. 10.

FIG. 13. Diplococcus in infiltrated area (*a*) shown in Fig. 12. Gram-Weigert. $\times 1000$.



FIG. 2



FIG. 3

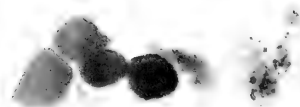


FIG. 5

FIG. 9



FIG. 11



FIG. 13

PLATE 2

FIG. 1. Section of the muscle excised from the left side of the patient's neck. Note the marked infiltration by connective tissue, the irregular staining and the atrophy of muscle fibers. Hematoxylin and eosin. $\times 60$.

FIG. 4. Rabbit (R 656) showing marked lacrimation of the left eye and swelling of the left side of the face forty-eight hours after the intravenous injection of streptococcus from the pulp of the extracted first upper left molar.

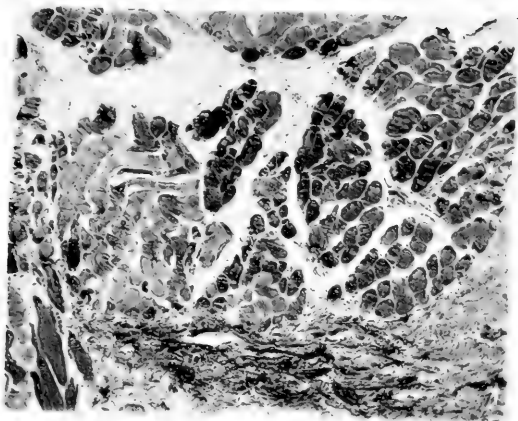


FIG. 1

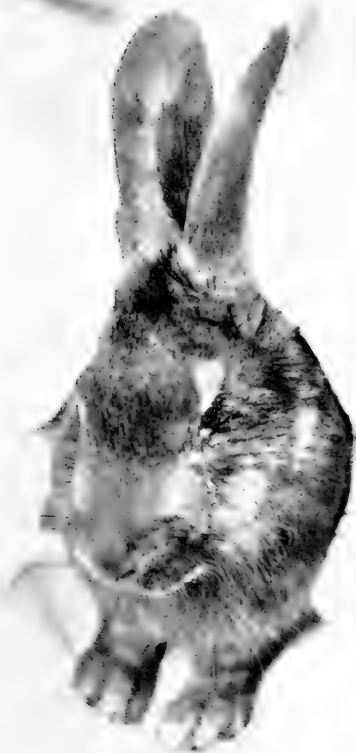


FIG. 4

PLATE 3

FIG. 6. Left lower jaw of rabbit (R 659) injected three days previously with 5 cc. of twenty-four hour tissue-ascites-fluid culture of the streptococcus from the tooth. Note the edema and hemorrhage in the periosteum opposite the root of the canine and surrounding the inferior dental nerve at the foramen of exit. $\times 2$.

FIG. 7. The pulp of the inferior canine and inferior dental nerve from dog (D 412) two days after an intravenous injection of the streptococcus isolated from the hemorrhagic pulp of the second upper left molar. Note the numerous large and small hemorrhages in the pulp of the left lower pulp and nerve. $\times 2$.

FIG. 8. Section of the pulp of the left lower canine shown in Fig. 7. Note the marked hyperemia and the large and small hemorrhages. Hematoxylin and eosin. $\times 60$.

FIG. 10. Section through the hemorrhagic area in the left inferior dental nerve, shown in Fig. 7. Note the marked edema and large and small hemorrhages and leucocytic infiltration of the sheath. Hematoxylin and eosin. $\times 60$.



Fig. 7



Fig. 10



Fig. 6



Fig. 8

PLATE 4

FIG. 12. Section of the dental pulp of the left upper molar in guinea pig (P 141) injected two days previously with the streptococcus from the pulp of the patient's tooth. Note the marked leucocytic infiltration and hemorrhage. Hematoxylin and eosin. $\times 240$.

FIG. 14. Section of the left triceps of guinea pig (P 155) injected two days previously with the streptococcus from the muscle of the patient. Note the marked hemorrhagic and leucocytic infiltration and separation of muscle fibers. Methylene blue and eosin. $\times 140$.

FIG. 15. Diplococcus in infiltrated area shown in Fig. 14. Gram-Weigert. $\times 1000$.

FIG. 16. Section of the left trapezius in rabbit (R 660) forty-eight hours after intravenous injection of the streptococcus from pulp of the left upper molar in rabbit shown in Fig. 4. Note the marked edema, leucocytic infiltration, hemorrhages and the disintegration of the muscle fibers. Hematoxylin and eosin. $\times 120$.

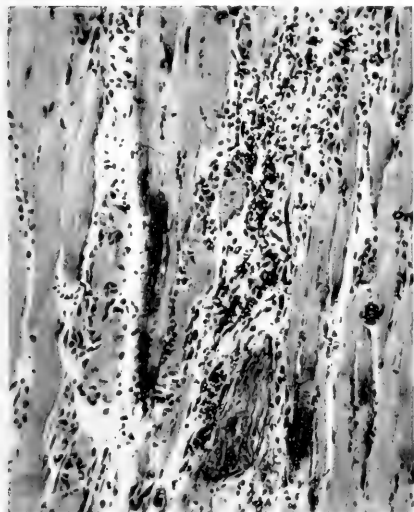


Fig. 14



Fig. 16

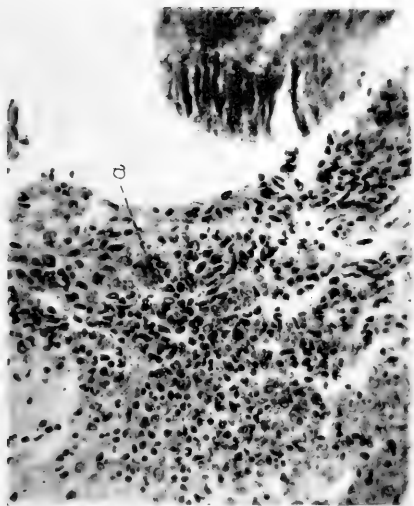


Fig. 12

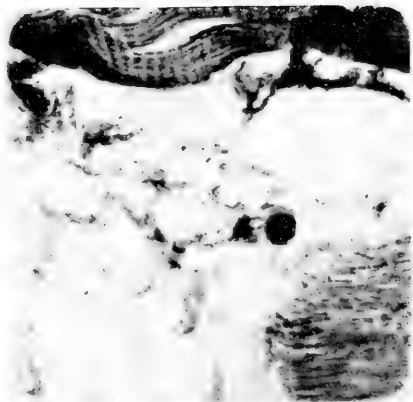


Fig. 15

PHAGOCYTOSIS OF ERYTHROCYTES DURING STAGE OF HYPERLEUCOCYTOSIS FOLLOWING INJECTION OF TYPHOID BACILLI

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In the course of a study of the leucopenia and subsequent hyperleucocytosis that follow the intravenous injection into the rabbit of typhoid bacilli, either living or killed, it was necessary to examine a large number of stained spreads of blood taken before the injection and at various intervals thereafter. It was during these examinations that the present instance of phagocytosis of erythrocytes in the peripheral blood was observed.

In general the leucocytic response in the blood of our rabbits was analogous to that obtained by other workers after similar injections. This reaction consists of two phases; the first a marked leucopenia which appears within a few minutes after the injection and reaches its low point within two hours. The count of white blood cells may drop from normal to as low as 1000 per cmm. and differential counts made at this point show this leucopenia to be in chief part the result of the almost complete disappearance from the blood, as obtained from the ear, of the polymorphonuclear amphophiles. This cell constitutes between 30 and 50 per cent of the circulating leucocytes in the normal rabbit but during the stage of marked leucopenia it is often present as less than 1 per cent of the total count. The other varieties of white cell also decrease in actual numbers but the percentage of small lymphocytes rises to 90 per cent or even higher. In several instances no cells other than small lymphocytes and very infrequent mast cells remain in the blood at the low point of the leucopenia. This is suggestive since mast cells

and small lymphocytes are the only types of white blood cells supposed to be devoid of all phagocytic activity.

The second phase of the reaction consists in a marked leucocytosis which reaches its maximum in from eighteen to twenty-four hours after the injection. In our series the highest count, 108,400, was obtained twenty-four hours after the injection of killed organisms into a non-immunized rabbit. In this leucocytosis the amphophiles again play the chief part and their percentage may rise as high as 85 to 90 per cent. Many of these cells appear to be young forms and there is some deviation to the left on the Arneth scale.

The occurrence of phagocytosis of erythrocytes in the blood stream was observed at the height of the leucocytosis in an animal that had received an injection of one-tenth of a twenty-four hour agar slant culture of *Bacillus typhosus* washed off in salt solution and heated at 60° for one hour. The usual leucopenia occurred and was followed by a leucocytosis which had risen, twenty-six hours after the injection, to 43,600. The differential count at this time was: Amphophiles 70 per cent, small lymphocytes 23 per cent, large mononuclears 4 per cent and Mast cells 3 per cent. This percentage of large mononuclears is much higher than is usual in the stage of leucocytosis, for as a rule this variety of white cell plays little or no part in the increased count. In the blood before injection the total count was 15,900 of which 7 per cent were large mononuclear cells. This figure is normal for the rabbit. In the stained preparations taken at this time it was observed that the majority of these large mononuclear cells showed evidence of active phagocytosis of the circulating erythrocytes. These macrophages could in no way be differentiated from the non-phagocytic mononuclear cells of the rabbit's blood. Many of them contained two or more erythrocytes and the engulfed cells showed every degree of digestion; in some instances merely a trace of the erythrocyte remaining in the center of a clear vacuole. One instance in the same preparation was observed of the phagocytosis of an erythrocyte by a polymorphonuclear amphophile, but there was no other evidence of phagocytosis by other forms than the large mono-

nucleated cells. Occasionally a lymphocyte or amphophile had been taken up by one of the macrophages.

Twenty-four hours later the leucocyte count had fallen to 13,800, the differential picture had become normal and almost all evidence of phagocytosis had disappeared. Infrequently, however, there could still be found large mononuclear cells containing digested erythrocytes. No subsequent observations were made on this animal as it was unfortunately destroyed before the stained spreads were studied nor was any phagocytosis of erythrocytes observed in the other animals that had been given similar injections.

Phagocytosis of erythrocytes occurs under a number of conditions. It is the physiological fate of the erythrocytes in many species in which, as Kyes (1) has emphasized, the phagocytosis is carried on by large fixed tissue macrophages chiefly in the liver and spleen. Foreign erythrocytes introduced into the circulation are eliminated through the agency of these macrophages in the central organs as shown by Levaditi (2), Cary (3) and others and no evidence of phagocytosis is to be found in the peripheral blood. On the other hand Hopkins (4) has reported a case of pernicious anemia in which after a transfusion, there occurred active phagocytosis of erythrocytes by the polymorphonuclear neutrophils of the circulating blood. *In vitro* phagocytosis of heterologous erythrocytes can be readily brought about under proper conditions by the polymorphonuclear leucocytes of a leucocytic cream or by endothelial cells obtained from ascitic fluid. These latter cells are also actively phagocytic for erythrocytes introduced into the peritoneal cavity.

In both pernicious anemia and typhoid fever, in which increased phagocytosis by the macrophages in the internal circulation occurs, there have been rare reports of the finding of similar cells in the peripheral blood. Van Nuys (5) has reported a case of fatal anemia with active phagocytosis of polymorphonuclear neutrophils and erythrocytes by macrophages resembling endothelial cells. Another case of fatal anemia reported both by Bartlett (6) and by Rowley (7) showed remarkable phagocytosis of both red and white cells by every variety of normal

leucocyte as well as by myelocytes and "plasma cells." Eichhorst (8) in a case of typhoid fever noted that for five days the peripheral blood contained large cells, which were actively phagocytic for the erythrocytes. He also quotes the finding in 1842 by Gullivier of "lymph cells" containing from one to six red blood cells in the circulating blood of a horse dying of phlebitis. Phagocytosis of erythrocytes has also been observed in leukemia.

The conditions under which the observation here reported occurred clearly differ from any of those mentioned above and no satisfactory explanation can be advanced. It is of interest, however, in view of the extensive phagocytosis of erythrocytes which takes place in typhoid fever that this instance of transitory peripheral phagocytosis of erythrocytes followed the injection of typhoid bacilli.

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CONVALESCENT TYPHOID SERUM IN THE TREATMENT OF TYPHOID FEVER¹

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Immuno-therapy in typhoid fever has been attempted in many ways. While we all realize that one of the most efficient forms of any immuno-therapy is by means of passive immunization or serum therapy (compare diphtheria, cerebro-spinal meningitis, etc.) no really efficacious serum has as yet been produced for the typhoid infection. Numerous steps have been taken but none have been crowned by success. An attempt was therefore made to transfer the active immunity present in convalescent typhoid patients to those ill with the disease by means of the blood serum. I selected for this treatment only those with the severest infections, very high temperature and marked toxemia; in fact at the time of admission to the hospital of each of the three cases, a most discouraging or hopeless prognosis was given to the family.

Case 1. Boy twelve years old, admitted in the second week of the disease. Very sick; muttering delirium; tremendous crop of rose spots; dry, red tongue. Spleen enlarged to four fingers breadth below costal margin. Was at the hospital for four days before serum was administered. Temperature ranged between 103° and 104°; pulse between 100 and 128; respirations between 28 and 52. Widal reaction was positive; blood culture was negative. The patient received 50 cc. serum subcutaneously on the fifth day of admission, 50 cc. on the sixth day, and 30 cc. on the seventh day. The serum was obtained from the blood of two convalescent typhoid patients in the same ward, one of whom had had a normal temperature for two days, the other had a normal

¹Read before the American Association of Immunologists, Washington, D. C., May 11, 1916.

temperature for ten days. The beneficial effect of the serum was distinctly evident. The general mental condition of the patient improved rapidly, the temperature began to decline so that it touched the normal mark but with a remission three days after the last serum injection, and remained normal six days afterward. The spleen became smaller and the boy made a rapid, uneventful recovery.

Case 2. Man thirty-two years old; admitted in the second week of the disease. Very sick; stuporous; difficulty of hearing with left ear. Large crop of rose spots. Moist tongue. Spleen enlarged to three fingers breadth below the costal margin. Was at the hospital for four days before the serum was administered. Temperatures ranged between 103° and 106° F. Pulse between 90 and 110. Respirations about 26. Widal was positive. Blood culture was positive. Received 100 cc. of serum intramuscularly on the sixth day and 100 cc. on the seventh day. The serum was obtained from two convalescent patients: one had had a normal temperature for ten days and the other for twelve days. I wanted to administer this serum intravenously but found that the patient's red blood cells were agglutinated by the donors' sera. In this case as in the first the favorable effects of the serum were readily observed. The general condition improved, the pulse came down and rose to 100 only twice, the temperature dropped about 4° in forty-eight hours and touched normal about five days after the second serum injection.

Case 3. Was the sickest of the group and the result was not as favorable. Man about thirty years of age; in the third week of the disease; a large husky sailor but completely overcome by toxemia; delirious. Temperature ranged between 104° to 105½°, pulse above 120 requiring stimulation; repeated very severe epistaxis requiring packing of the anterior and posterior nares and also repeated intestinal hemorrhages; Widal positive. 100 cc. of serum were administered intravenously on the third day after admission; 75 cc. intravenously on the fifth day; and 100 cc. subcutaneously on the seventh day; the serum was obtained from two convalescent patients whose bloods were tested for agglutination and hemolysis against the blood of the patient. One of the donors belonged to the same group as the patient, and therefore his serum was administered intravenously; the serum from the other, who did not belong to the same group, was administered subcutaneously. In this case also, beneficial effects from the serum were noticeable. The mental condition of the patient improved and the temperature came down but his anemia was very marked; his general condition

remained very weak and he took very little nourishment. A large spreading abscess of the scrotum and adjacent gluteal region developed, which required extensive surgical interference. Several days afterward he died.

Nicolae and Conseil (1) similarly treated five patients. They claim that the duration of the disease was not shortened, although certain nervous symptoms seemed to be improved. They used only about 130 cc. of serum for each patient, which is less than the quantities used above. It is a known rule for serum therapy in general that an excess of serum must be employed. This fact makes one realize that the above described procedure could hardly become general in practice. Typhoid convalescents as a rule are in need of all the blood serum they possess. In special instances, however, this mode of therapy should be considered.

CONCLUSION

The serum from convalescent typhoid patients has been employed with distinct benefit in three very severe acute typhoid cases.

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THE COMPLEMENT FIXATION TEST AFTER PROPHYLACTIC TYPHOID IMMUNIZATION AND A COMPARISON WITH THE AGGLUTINATION TEST¹

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In a previous paper (1), the author demonstrated that practically all typhoid fever patients develop complement fixation bodies in their blood sooner or later during the course of the disease. These antibodies persist during convalescence and for a long time afterward. The next question of interest was whether these bodies are also present in the blood of individuals that have been artificially immunized against typhoid fever. While the agglutinins and bacteriolysins in inoculated cases have been carefully studied, no detailed statistics can be found in the literature referring to the complement fixation bodies. Possibly this may be explained by the more complicated technique necessary for the demonstration of these bodies; or (as is mentioned by Russel) their presence was taken for granted on account of the finding of agglutinins and bacteriolysins, as the latter belong to the same class of antibodies; i.e., amboceptors, as do the complement fixatives. A closer investigation of the problem was therefore undertaken.

Keeping in mind the observations of Teague and Torrey, (2), Raskin (3), Garbat (4), etc., that various strains of the same bacteria often differ as to their specific antibodies, the writer has always deemed it advisable to employ a polyvalent typhoid vaccine made from at least seven or eight different virulent strains. These had been isolated from the blood of hospital patients. The vaccine is prepared and standardized according

¹Read before the American Association of Immunologists, Washington, D. C., May 11, 1916.

to the method of Wright. As a pupil of the English school, the writer, during the years 1910-1912, followed the English method of prophylactic inoculation; i.e., two injections of larger doses were given, the first of 1,000,000,000 and after ten days the second dose of 2,000,000,000 microorganisms instead of the three smaller doses. Since then the method of three injections (500,000,000, 1,000,000,000, 1,000,000,000), was employed.

Parallel examinations of the agglutination and complement fixation tests were made. The former was carried out by the hanging drop method with increasing dilutions of the serum; (each dilution being mixed in equal parts with a twenty-four hours broth culture of typhoid bacillus (Pfeiffer strain) and examined microscopically at the end of half an hour.) The "Pfeiffer" strain (one of the seven strains used in making up the vaccine) was chosen for the agglutination test on account of its very marked motility and little tendency towards artificial clumping.

The details of the *complement fixation* test have been outlined in a former paper (1). One point must be mentioned. Since it had been observed (Raskin, Garbat) that complement fixation bodies produced by certain strains of the typhoid bacillus will react only when mixed with an antigen made from the same strains, the antigen employed in our tests was prepared from the same seven strains used in making up the vaccine for inoculation.

Thirty-eight cases were injected twice (1000 M. and 2000 M. respectively).

In 32 cases only one examination for complement fixation was possible: 9 cases were examined 1 year after inoculation; 5 cases were examined 2 to 3 months after inoculation; 8 cases were examined 1 to 2 months after inoculation; 10 cases were examined within the first month after inoculation. The complement fixation test was *negative* in practically all instances. Two gave a doubtful reaction. On the other hand the agglutination test was strongly positive in all but two instances one year after inoculation and ranged from 1-200 to 1-4000.

Naturally, one may raise the question, in view of later findings, that some of these patients had possibly developed comple-

ment fixation bodies which persisted only for a short period but had disappeared at the time of my examination long after inoculation. The great majority, however, (23 cases) were examined within the first three months after the inoculation.

Nevertheless, to answer this question the second series of six cases was selected where several examinations were made in the same individual at various intervals after the inoculations in order not to miss a transient positive reaction. Of this group only one developed a distinctly positive (3+) complement fixation test. The other five remained negative although the agglutination titer was as high as 1-5000.

From the above results; i.e., the almost uniform negative findings after prophylactic immunization (two inoculations method) and from the results of my former investigation, that all typhoid fever patients develop a positive complement fixation, it seemed reasonable to conclude that the complement fixation test may prove of assistance in diagnosing or excluding typhoid fever in persons sick with a suspicious fever who had been vaccinated some time past and still had a positive Widal reaction. A positive fixation test would speak in favor of an active typhoid; a negative test would speak against typhoid and favor the positive Widal as being dependent upon the prophylactic immunization. These experiments were concluded in 1914.

Felke (5) came to absolutely the same conclusion after examining 39 persons vaccinated against typhoid. The agglutination test gave nearly constant positive findings while the complement fixation test was constantly negative. On the other hand, in nine typhoid fever cases and in a few convalescing ones, both tests were positive. My experiments, which led me to the same opinion, were completed one year before this German article, and a radical conclusion would have been as erroneous as Felke's had I published my results at that time and not undertaken still another series of cases in whom the American method of *three inoculations* was employed. Fourteen cases were immunized by three injections of the polyvalent vaccine at intervals of one week. Seven developed no complement fixation test, although in some the agglutination was present as high as 1-10,000.

Seven developed a positive complement fixation test. (Nothing less than 2+ was considered positive.)

- (a) In 2 the reaction persisted for 6 months after inoculation.
- (c) In 2 the reaction persisted for 2 weeks after inoculation.
- (b) In 1 the reaction persisted for 5 weeks after inoculation.
- (d) In 2 the reaction was present 1 week after inoculation but repetition of the test was impossible.

Thus it is seen that the complement fixation test did appear in about 50 per cent of cases inoculated three times with a polyvalent vaccine. There is however no regularity for its appearance. The two cases in whom it lasted six months were exceptionally robust individuals, one the porter of the laboratory, the other a member of the house staff, formerly a star ball player.

Still another group of 21 nurses were examined who also had received three injections but of a vaccine that was supplied by the Health Department of the city of New York and made from the standard Army "Rawling" strain. None developed a complement fixation reaction and their agglutination titer was rather low in comparison with the two former groups inoculated with the polyvalent hospital vaccine.

DISCUSSION

The negative complement fixation test after prophylactic typhoid inoculations is interesting from several standpoints:

1. The complement fixation bodies do not run parallel with the agglutinins. The latter are apparently more easily stimulated and persist for a longer time. An analogy may be found in prophylactic meningococcus vaccination. There too the total increase of complement fixatives was not as high as agglutinins (Sophian (6)).
2. It has been shown that prophylactic immunization is regularly followed by the production of bacteriolysins. The latter are amboceptors too, but apparently not identical with complement fixation bodies. This conforms with the findings of Kolmer (7).

3. The complement fixation bodies cannot be taken as an evidence or an index of immunity; clinical experience has shown that prophylactic injections protect the very great majority of individuals. I had already shown in a previous paper that a serum may possess a high degree of curative properties and yet contain no complement fixation bodies (8).

4. And most important, the reaction or perhaps we may say the immunity after an attack of typhoid fever is apparently different from the artificially acquired immunity, since the former is in almost all instances associated with the production of complement fixation bodies. The possible difference in reaction may perhaps be accounted for by the fact that during the infection with the typhoid bacillus one is being inoculated so to say, first, with live bacteria instead of organisms killed by heat, and secondly, with a comparatively much greater total number of bacteria than in prophylactic immunization. The positive complement fixation test in typhoid carriers speaks in favor of this explanation.

The fact that *animals* inoculated with dead typhoid bacteria develop a strong complement fixation test may possibly also be explained on the basis of greater quantity of antigen; for, when animals are immunized, very many more bacteria in proportion to the body weight of the animal are injected than in the prophylactic immunization of human beings. Actual experiments proving the relationship between the number of antibodies produced and the number of bacteria injected in proportion to the body weight of the animal are under way.

Why, however, one individual should react by the production of complement fixation bodies sufficient to give a strong reaction and others not, cannot be definitely answered. We all have had similar experience in immunization of the lower animals with any antigen, red blood cells, bacteria or proteins, etc. Under the same precautions some would develop numerous antibodies, others less and some none. Similar conditions exist also in whooping cough. About 40 to 50 per cent of whooping cough patients develop complement fixatives, while prophylactic immunization with vaccine produces hardly any (9).

SUMMARY

1. In contrast with the strong agglutination test, a positive complement fixation test after prophylactic typhoid immunization is not as regular an occurrence, as it is during or after typhoid fever. This point may be of aid in deciding for or against the diagnosis of typhoid fever in an inoculated individual still having a positive Widal and ill with a suspicious typhoid but negative blood culture.

2. A positive complement fixation test was obtained most often after three injections with a polyvalent vaccine; two injections with this same vaccine or three injections with the single strain vaccine (Rawling) gave hardly any complement fixation.

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EVIDENCE AS TO THE INDEPENDENCE OF HEMOLYSINS AND PRECIPITINS

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In the absence of chemical methods affording complete isolation and analysis of antibodies, our knowledge concerning the structure and properties of these substances is at best but approximate.

Although theoretically the injection of a host with a mixture of several proteins may well result in the production of multiple antibodies, the extent to which this actually occurs in a given instance has not been more than partially determined. Cytolysis, cyto-agglutination, precipitation, toxin neutralization and opsonification, we recognize as among the various evidences of antibody activity, but to just what extent each of these activities is due to an independent and distinct antibody is far less defined than the generalizations of certain theories concerning immunity might lead us to conclude.

As bearing upon this problem I wish to report certain experimental results, which suggest the co-existence in the same serum of two distinct antibodies, each producing its own type of reaction.

The results were incidentally observed in two rabbits serving as controls for a series of experiments not here included. The animals (A and B) were injected intraperitoneally with 1 cc., 2 cc. and 2.5 cc. of fresh beef serum on the first, thirteenth and twenty-second days, respectively. Both animals were injected at the same time and with the same material. Prior to the injections on the first and thirteenth days and also on the twenty-fifth day, both animals were bled (10 cc.) and tests made for

antibody content. The sera were tested primarily for their specific precipitating action for beef serum, but inasmuch as v. Dungern (1), Tschistowitsch (2), and Morgenroth (3) have shown that the injection of foreign serum may produce a specific hemolysin for the erythrocytes of the same species, tests were also made as to the presence of a hemolysin specific for beef corpuscles.

The precipitin determination was made by adding decreasing amounts of the "immune" serum to tubes each containing 0.2 cc. of beef serum and sufficient 0.85 per cent NaCl solution to bring the total volume of each tube to 2 cc. The hemolysin determinations were made by adding decreasing amounts of the inactivated "immune" rabbits' sera to tubes each containing 1 cc. of a 5 per cent suspension of beef erythrocytes and 0.05 cc. of active guinea pig serum in a total volume of 2 cc. In both types of test the tubes were placed at 38°C. for two hours and at 12°C. for twenty-two hours; the readings being made at the close of the latter period. Tabulated, the results appear as follows:

TABLE 1
Precipitin test

RABBIT SERUM	BEEF SERUM 0.2 CC. IN EACH TUBE					
	First day		Thirteenth day		Twenty-fifth day	
	Rabbit A	B	A	B	A	B
cc.						
0.5	0	0	+++	0	+++	0
0.375	0	0	++	0	++	0
0.25	0	0	++	0	++	0
0.175	0	0	+	0	+	0
0.125	0	0	+	0	+	0
0.075	0	0	?	0	+	0
0.05	0	0	?	0	+	0
0.0375	0	0	0	0	+	0
0.025	0	0	0	0	?	0
0.0175	0	0	0	0	0	0
0.0125	0	0	0	0	0	0
Control						
0.000	0	0	0	0	0	0

TABLE 2
Hemolysin test

RABBIT SERUM	1 CC. 5 PER CENT BEEF ERYTHROCYTES + 0.05 CC. GUINEA-PIG SERUM			
	First day		Twenty-fifth day	
	Rabbit A	B	A	B
cc.				
0.5	0	0	Complete	Complete
0.375	0	0	Complete	Almost complete
0.25	0	0	Marked	Marked
0.175	0	0	Medium	Medium
0.125	0	0	Medium	Medium
0.075	0	0	Medium	Medium
0.05	0	0	Medium	Medium
0.0375	0	0	Slight	Slight
0.025	0	0	Slight	Slight
0.0175	0	0	Trace	Slight
0.0125	0	0	0	0
Controls				
0.000	0	0	0	0
Rabbit serum with- out guinea-pig serum 0.5 cc.	0	0	0	0

As tabulated above the experiments show that the parallel injection of beef serum into the two rabbits resulted very differently in the production of a precipitating "immune" serum. The serum of rabbit A displayed a distinct precipitating action on the thirteenth day as the result of a single injection and an increased precipitating action on the twenty-fifth day as the result of three injections. In contrast, the serum of rabbit B, showed no precipitating action at any time whatsoever (table 1).

On the other hand when these sera were tested for hemolytic action, it was found that in regard to this action the two sera were of approximately the same value (table 2).

Stated in general terms then the parallel injection of beef serum into two rabbits resulted in the one instance (A) in a specific hemolytic serum; in the other instance (B) in a specific hemolytic and precipitating serum.

In explanation of such an occurrence it might be conceived that the newly formed hemolytic substance in the one instance was distinctly different from that in the other; namely, that in animal A the substance that produced hemolysis had no precipitating power, whereas in animal B a different substance, produced both hemolysis and precipitation. The probability of such an explanation appears slight, however, when the specificity of antibody reactions is taken into consideration. In other words, where the reaction is so specific as is antibody hemolysis it appears improbable that in two animals of the same species, two specific hemolytic substances would be produced which so far differed from one another as to have a powerful precipitating action in the one instance and none in the other.

An alternative explanation appears more probable; namely, that in both animals the same hemolytic substance was produced, but that in addition, in one of these animals (B) there was also produced a distinct precipitating substance. It is on the basis of this probability that the above experiments indicate the independence of two substances producing respectively hemolysis and precipitation.

I wish to express appreciation of the interest of Professor Preston Kyes in whose laboratory this work was pursued.

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ENDOTHELIAL OPSONINS¹

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If virulent pneumococci are injected intravenously in sufficiently large doses into normal rabbits, they will remain indefinitely in the circulating blood. Counts made at the end of thirty minutes and one hour, for example, will usually show about 25 per cent of the total number of injected pneumococci still present in the blood-stream. If, however, the same doses of pneumococci are injected intravenously into actively immunized rabbits all but one or two per cent of them will disappear from the circulation in from five to seven minutes. The blood usually becomes sterile in from ten to fifteen minutes.

This rapid and complete disappearance of injected pneumococci from the blood of actively immunized rabbits has been studied by numerous investigators, particularly by Bull (1) of the Rockefeller Institute. Bull attributes the disappearance to intravascular agglutination of the injected pneumococci, followed by a removal of the agglutinated masses by a process of mechanical filtration by the capillaries of the organs through which the blood passes.

A year ago Kitagawa (2) working in our laboratory showed, however, that the process of agglutination and mechanical filtration emphasized by Bull is not the only factor operative in this removal.

Kitawaga worked with a highly virulent first-generation growth of pneumococci from heart-blood, the growth taking place in the presence of rabbit serum. These pneumococci were practi-

¹ Presented before the American Association of Pathologists and Bacteriologists, Washington, D. C., May 9, 1916.

cally inagglutinable in the serum of immunized rabbits, and also inagglutinable in the blood-stream. This latter he showed by isolating samples of infected circulating blood between ligatures placed about the larger blood vessels. Plates and smears made from the ligated samples, at the end of fifteen, thirty, and sixty minutes, usually showed no appreciable diminution in the pneumococcic count, no agglutination, and little or no phagocytosis. Nevertheless, these high-resistant, inagglutinable pneumococci disappeared promptly and completely from the circulation when injected intravenously into immune rabbits.

Kitawaga concluded from his observation that the disappearance could not be due to a purely mechanical filtration of agglutinated masses by the tissue capillaries, but that it necessitated some active biological coöperation of the capillary cells.

We have endeavored to determine the nature of this active cellular process.

Methods. We have applied to this determination the method of perfusion of isolated organs and tissues. Mixtures of pneumococci, active and inactive sera, defibrinated blood, etc. in Ringer's solution, have been repeatedly perfused through organs; plates and smears made at the end of each passage and compared with plates and smears from control samples of the same mixtures kept at incubator temperature and not passed through the tissues. At the end of the perfusion, smears and histological preparations were made from the perfused tissues.

The perfusions in all cases have been made at body temperature, with a pressure and rate of flow approximately equal to the normal blood pressure and rate of flow in the organ or group of tissues studied. The volume of the perfusion fluid has usually been 75 cc. to 125 cc., an amount approximately equal to the total blood-volume of the rabbit tested. From 1 cc. to 2 cc. of a twenty-hour broth culture of pneumococci have usually been used in the perfusions.

PERFUSION OF LIVERS

Method. Afferent cannula in portal vein, efferent cannula in vena cava immediately above diaphragm; ligature of remaining portal tissues and of vena cava immediately below liver; perfusion in situ under 20 to 30 cm. H₂O pressure.

If the liver of a normal rabbit is washed free from its contained blood by a preliminary perfusion with Ringer's solution, and is then repeatedly perfused with a sample of Ringer's solution containing a known number of pneumococci, no diminution in the pneumococcic count of the perfusion fluid is observed, even after a dozen passages through the liver (A, fig. 1).

If the liver of an actively immunized rabbit is similarly perfused, the perfusion fluid usually becomes sterile after from two to five passages through the liver (B, fig. 1).

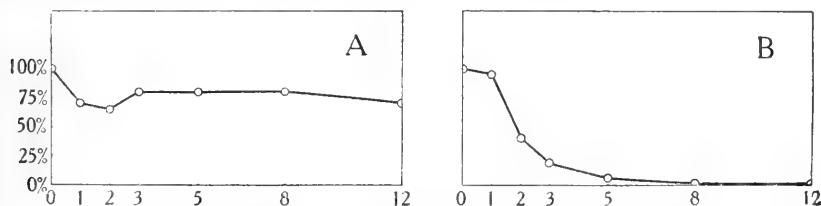


FIG. 1. COMPARISON OF NORMAL AND IMMUNE LIVERS

A, normal liver, B, immune liver. Preliminary washing with 500 cc. Ringer's solution. Subsequent perfusion with 75 cc. Ringer's solution containing 2 cc. of a twenty-hour broth culture of pneumococcus. Heavy broken lines show changes in the number of pneumococci in the perfusion fluids at the end of each passage through the liver. In interpreting these results, it is necessary to take into account the dilution of the perfusion fluids by the residual Ringer's solution left in the livers after the preliminary washing. The volume of this residual fluid is approximately 20 cc.

Smears and histological preparation made from the perfused immune liver now show numerous pneumococci adherent to the capillary endothelium. Few if any agglutinated masses are seen, and little or no endothelial phagocytosis.

Phenomenon analysed. The addition of from 1 per cent to 10 per cent normal rabbit's serum to the perfusion fluid, causes no appreciable retention of the pneumococci by normal livers. The addition of similar amounts of immune serum, however, causes the pneumococci to be retained quantitatively by the normal liver (fig. 2).

The amount of immune serum necessary to cause this retention is very small. Parallel tests with one serum, for example (fig. 3), showed a practically quantitative retention of the pneu-

mococci on the second passage through the normal liver with 1 : 1000 immune serum, a practically quantitative retention by the fifth passage with 1 : 10000 immune serum, and a two-thirds retention by the twelfth passage with 1 : 100,000 immune serum. The agglutination titer of this serum was 1 : 100 with second-

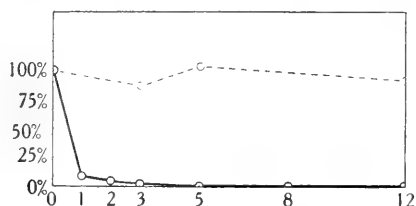


FIG. 2. ACTION OF IMMUNE SERUM

Normal liver. Perfusion fluid: 1:100 immune serum in Ringer's solution. Heavy broken line shows changes in the pneumococcic count of the perfusion fluid at the end of each passage through the liver. Dotted line shows parallel counts of a control sample of same fluid kept at incubator temperature but not yet passed through the liver.

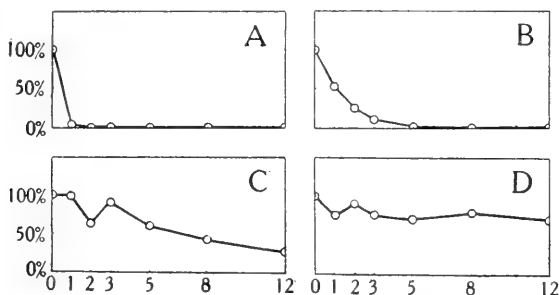


FIG. 3. TITRATION OF IMMUNE SERUM

Normal livers. Perfusion fluids: A, 1:1000 immune serum, B, 1:10,000 immune serum; C, 1:100,000 immune serum; D, Ringer's solution (control).

generation pneumococci; first-generation pneumococci inagglutinable.

The retention of the pneumococci by the immune liver (fig. 1), therefore, could be wholly accounted for by the existence of a small amount of residual immune serum in the liver after the preliminary washing with Ringer's solution. The fact that the

pneumococci passed through the liver practically quantitatively on the first passage of the perfusion fluid, but were retained in large numbers on the second passage, would indicate that the retention took place only after an admixture of the perfusion fluid and the residual serum.

Active principle. The active principle of the immune serum responsible for this retention is thermostable. Serum heated to 60°C. for thirty minutes does not appreciably lose its power to cause this retention (A, fig. 4). The power is only partially lost after heating to 75°C. for thirty minutes (B, fig. 4).

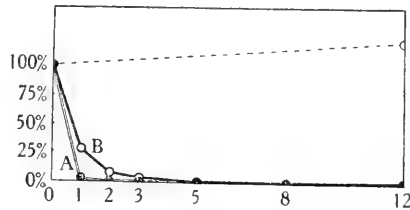


FIG. 4. THERMOSTABILITY OF ACTIVE PRINCIPLE

Normal livers. Perfusion fluids: A, 1:100 immune serum heated to 60°C. for thirty minutes; B, 1:100 immune serum, 75°C., thirty minutes. Dotted line, incubated controls.

Pneumococci exposed to subagglutinating dilutions of immune serum, then freed from serum and repeatedly washed by centrifugation, and then suspended in Ringer's solution, are retained quantitatively by normal livers (fig. 5).

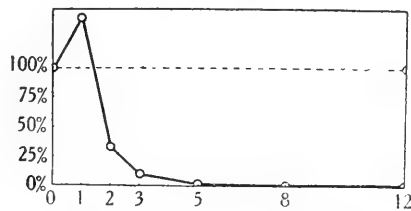


FIG. 5. SENSITIZED PNEUMOCOCCI

Normal liver. Pneumococci sensitized by exposure, at 37°C., for thirty minutes to 1:400 inactivated immune serum. The agglutination titer of this serum was less than 1:100. Perfusion fluid, Ringer's solution. Dotted line, incubated control.

The fact that these sensitized pneumococci pass through the liver quantitatively on the first passage of the perfusion fluid, but are largely retained on its second passage, is taken to indicate that, in addition to the specific thermostable substance, a relatively large amount of some normal serum component is necessary for the retention. This normal serum component is apparently furnished by the residual serum of the liver.

The serum component responsible for the pneumococcic retention is, therefore, presumably an opsonin or bacteriotropin so altering the pneumococci as to cause their adhesion to the capillary endothelium. For lack of a better term, we have referred to this substance as an *endothelial opsonin*.

PERFUSION OF EXTRA-HEPATIC TISSUES

Methods. Intestines. Afferent cannula in principal mesenteric artery at junction with aorta, efferent cannula in portal vein; double ligature of duodenum, rectum, and remaining mesenteric tissues; incision between ligatures with cautery; intestines removed from body and floated in bath of Ringer's solution, at 38 C.; perfusion under 80 to 120 cm. H₂O pressure.

Kidney. Cannulae in renal artery and renal vein near junction with aorta and vena cava; perfusion in situ, 80 to 120 cm. H₂O pressure.

Lungs. Afferent cannula in arch of pulmonary artery; immediate preliminary perfusion in situ; ligature of aorta and vena cava below heart, and of all ascending blood vessels; inflation of lungs and ligature of trachea; removal and suspension of heart and inflated lungs; incision of left ventricle for escape of perfusion fluid; perfusion under 40 to 60 cm. H₂O pressure.

Hind quarters. Incision of abdominal wall with cautery; double ligature and incision of rectum with cautery; cannulae in aorta and vena cava immediately below origin of renal vessels; double ligature of ascending collaterals; severance of hind-quarters from rest of body with cautery; perfusion under 80 to 120 cm. H₂O pressure.

Similarly to the findings with the liver, pneumococci suspended in Ringer's solution or in dilute (1 per cent to 10 per cent) normal rabbit serum, can be repeatedly perfused through the isolated lungs, kidney, intestines, and hind-quarters of a normal rabbit without appreciable retention by these organs (light lines, fig. 6).

In contrast with the liver findings, however, the addition of (1 per cent) immune serum to the perfusion fluid will cause no appreciable retention of the pneumococci by these organs (*heavy lines*, fig. 6).

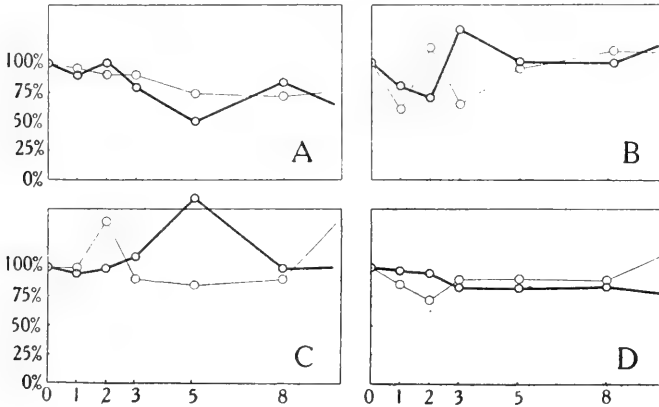


FIG. 6. PERFUSION OF EXTRA HEPATIC TISSUES

A, lungs; B, kidneys; C, intestines; D, hind quarters. Perfusion fluid (heavy line):—1: 100 immune serum. Light line, parallel control perfusions with 1: 100 normal serum.

The endothelial opsonin, therefore, is apparently operative mainly or solely with the endothelial cells lining the liver capillaries (spleen and bone marrow not yet studied).

DISTRIBUTION OF PNEUMOCOCCI IN ANIMAL BODY

If (60 per cent to 100 per cent) defibrinated normal rabbit blood is used as the perfusion fluid, slight but distinct deposits of pneumococci are made in all normal tissues. The deposit is most marked in the liver, where it may amount to as much as 15 per cent to 25 per cent of the pneumococci on each passage of the perfusion fluid through the organ (A, fig. 7).

The addition of a minute dose of immune serum to the defibrinated blood will cause the pneumococci to be deposited quantitatively in the normal liver (B, fig. 7).

The opsonin is therefore operative under the usual conditions existing in the animal body.

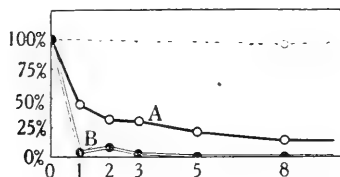


FIG. 7. ACTION OF DEFIBRINATED BLOOD

Normal livers. Perfusion fluid: A, Defibrinated blood; B, defibrinated blood + 1:100,000 immune serum (compare C, fig. 3). Dotted line, incubated controls.

SUMMARY

By the application of perfusion methods, an opsonin or bacteriotropin can be demonstrated in antipneumococcic serum, so altering pneumococci as to cause their adhesion to the endothelial cells lining the hepatic capillaries.

This opsonin is operative with the liver capillaries in many hundred times the dilution necessary to cause agglutination of the pneumococci, but is practically inoperative with the extra-hepatic capillaries.

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THE RELATION OF THE TYPHOIDIN SKIN REACTION TO IMMUNITY IN TYPHOID FEVER

ANAPHYLACTIC SKIN REACTIONS IN RELATION TO IMMUNITY. I

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Intimately associated with the earliest investigations and discoveries concerning agglutinins and bacteriolysins and being one of the first diseases in which active immunization by means of a bacterial vaccine was tried on a broad scale, typhoid fever has long been the subject of numerous investigations on the nature and mechanism of its immunity and particularly that which is known to follow efficient active immunization by means of a vaccine.

Since 1896 a large number of different vaccines prepared after various methods and regarded as possessing superior immunizing power, have been advocated in the prophylaxis of typhoid fever. While it has been fully realized that time alone could definitely prove the real value of the various vaccines and methods of immunization according to the protection afforded as compared to the incidence of the disease among the non-immunized living under the same conditions, many investigations have been made by serum studies *in vitro* and animal experiments *in vivo* with the purpose of determining the degree of the immunity response and the relative value of the different vaccines.

Following the discovery of von Pirquet of the cutaneous tuberculin reaction and its diagnostic value in this infection, similar

¹ Presented before the meeting of the American Association of Immunologists, May, 1916.

studies were made in other diseases among which typhoid fever was the earliest. These investigations were conducted with the main or sole purpose of determining the diagnostic value of ophthalmic and cutaneous anaphylactic tests in this disease, and with various results and opinions regarding their specificity and value. Chantemesse (1) observed characteristic inflammatory symptoms following the instillation of typhoid-bacilli extract into the eyes of patients suffering from typhoid fever. Kraus (2) and his associates found that normal individuals gave similar reactions and that typhoid fever patients reacted to bacterial extracts other than those of the typhoid bacillus. Zupnik (3), Link (4), Deehan (5), Floyd and Barker (6) and Austrian (7) have reported favorably upon the diagnostic value of cutaneous or ophthalmic reactions with monovalent or polyvalent anaphylactogens of typhoid bacilli prepared in various ways; Wolff-Eisner (8), Goodman and Sutter (9) and Chauffard and Troisier (10) have reported unfavorably upon their value and specificity. Owing to the greater ease of diagnosis of typhoid fever by means of blood cultures and agglutination tests and a disinclination of the profession to adopt the anaphylactic tests and particularly the ophthalmic test, these reactions have not been widely used in diagnosis.

More recently Gay and Force (11) have greatly renewed interest in this subject by advocating the skin test as a means of determining defensive activity following typhoid fever or active immunization by means of vaccines. Their first work was conducted with a "typhoidin" prepared in the same manner as Koch's old tuberculin by cutaneous inoculation; later Gay and Claypole (12) prepared typhoidin by precipitating the solution with alcohol, washing the precipitate with alcohol and ether, drying in a vacuum and suspending the resulting powder in phenolized normal salt solution which was injected intracutaneously and applied cutaneously; a control powder was prepared from broth and used in the same manner. With this skin test Gay and his associates have studied the relative value of various vaccines and regard the anaphylactic reaction as indicative of a state of immunity. Nichols (13) has questioned the

value of the anaphylactic skin test as an index of immunity and regards the reaction as indicating nothing more than sensitization to typhoid protein which is apparently less lasting and less specific than the true immunity to this infection. He bases this opinion on the fact that in his experience the typhoidin skin test gave fewer positive reactions (75 per cent) than generally expected, as about 90 per cent of persons who have had typhoid fever are immune for many years or even for the balance of life. Furthermore, according to Nichols, experience has shown that protection following typhoid fever is of longer duration than is indicated by the typhoidin test and while a large percentage of persons who have had typhoid fever or have been immunized with typhoid vaccine react to paratyphoidin recent experiences and statistics particularly in Europe have indicated that these persons are not immune to paratyphoid fever. Kilgore (14) has reported favorably upon the value of the typhoidin cutaneous test; Austrian and Bloomfield (15) found that the test failed to furnish data by means of which it was possible to differentiate between those who had neither typhoid fever nor had received the vaccine and those who had either had the disease or had been immunized.

Our immediate interest in this subject, in so far as this communication is concerned, has reference to the typhoidin anaphylactic reaction as an index of typhoid immunity as part of a series of studies (16, 17, 18) upon the subject of anaphylactic skin reactions in relation to defensive activity. The intracutaneous typhoidin test has been conducted with persons who have never had typhoid fever nor the vaccine; persons who have had the fever and no vaccine and persons who have never had the fever but who had received vaccine. The sera of a number of persons belonging to each of these groups were collected aseptically and submitted to bactericidal, agglutination and complement fixation tests. At the present time animal experiments consisting in the immunization of rabbits until a cutaneous anaphylactic response to typhoidin could be elicited, followed by a test of immunity against a living pathogenic culture injected intravenously and into the gall-bladder, have yielded results too irregular and inconstant to be of value at present and the fol-

lowing report aims briefly to record the results of experiments *in vitro*.

Many investigators have reached the conclusion that *in vitro* studies on the serum antibodies in typhoid fever are of little or no value as an index of immunity and particularly as a gauge of the immunity response after active immunization. Töpfer and Jaffe (19) found bactericidal substances present during and after typhoid fever, but the results varied with the technic. Tests after Neisser's method *in vitro* showed that the sera of convalescents were highly bacteriolytic, while *in vivo* tests after the method of Pfeiffer showed less bactericidal power; also the sera of paratyphoids showed strong bacteriolytic power over *B. typhosus in vivo*, but this activity was not apparent *in vitro*. Korte and Steinberg (20) found bacteriolysins present during and after typhoid fever which gradually diminished with the agglutinins. Stern and Korte (21) found that a high bacteriolytic content of a serum as determined by Neisser's method, does not necessarily indicate an immunity as one of their patients whose serum possessed a marked degree of bacteriolytic activity suffered a relapse. Klein (22) regards the role of agglutinins and bactericidal substances as doubtful and lays most stress upon the opsonins and phagocytosis in the immunity of typhoid fever. Russel (23) claimed that it was illogical to gauge immunity according to the detected presence of specific antibodies since agglutinins, opsonins and bacteriolysins disappear from the sera after typhoid fever in a few months and yet immunity lasts for several years if not for life. Leischmann (24) in his Harben lectures, laid most stress upon an increase in phagocytic activity as the important phenomenon in typhoid immunity and that this may be present when the bactericidal and agglutinin content is low and scarcely appreciable. Broughton-Alcock (24) likewise subscribes to the importance of phagocytosis by polymorphonuclear and endothelial cells and minimizes the role of agglutinins and bacteriolysins. Weston (25) found the sera of twenty immunized persons containing large amounts of bacteriolysin as measured *in vitro*, but has not expressed an interpretation of the significance to be attached; Moon (26) and many others have noted

an increase of agglutinins after active immunization, but generally state that the concentration of agglutinin in the body fluids does not run parallel with actual immunity. Gay and Claypole (27) lay particular stress upon the role of leucocytosis in resistance to typhoid fever and recovery from this infection and minimize the role of serum antibodies. These investigations have tended to minimize the role of agglutinins and bacteriolysins in typhoid immunity, but it does not appear to us that they can be dismissed entirely. While agglutinin itself has probably no destructive effect upon typhoid bacilli, it may act in the nature of a preparator for bacteriolysis and in this connection the agglutinins in general have received added importance as the result of Bull's recent studies in pneumonia showing intravascular agglutination. While not all persons react to typhoid vaccine with the formation of agglutinins, just as individual cases of typhoid fever may show but slight increase in typhoid agglutinin, the majority of persons do react with the formation of this antibody, and it has generally been regarded as an index of reaction and presumptive evidence that other antibodies may be present.

METHOD OF STUDY

Typhoidin tests. Cutaneous inoculation with a typhoidin and its control fluid prepared in the same manner as Koch's old tuberculin as described by Gay and Force, yielded unsatisfactory results. All of the tests reported in this paper were conducted with the powder typhoidin and a control powder kindly furnished us by Professor Gay. A portion of these powders was suspended in sufficient normal salt solution containing 0.25 per cent tricresol so that 0.1 cc. contained 0.001 or 0.0005 mgm. These amounts were injected intracutaneously after preliminary cleansing of the skin of both arms.

Cutaneous tests were also conducted at the same time with a 1 per cent suspension of each powder in tricresolized saline solution.

All tests were made within two weeks after receipt of the typhoidin and the suspensions kept at a temperature near the freezing point during this time.

Typhoidin reactions. The majority of the cutaneous tests were negative with both typhoidin and the control powders; in cases where reactions occurred the area of erythema was slight and in only four

instances out of forty-two inoculations could the reaction be regarded as definitely positive at the end of twenty-four or forty-eight hours on the basis of wider erythema and greater edema at the site of typhoidin inoculation.

Extensive reactions usually followed the intracutaneous inoculations. At the end of forty-eight hours both the typhoidin and control sites showed a wide area of faintly outlined erythema or cutaneous "blush" which was difficult to measure and accompanied by much edema, heat and pain upon movement of the arm. About the needle puncture there was usually a small, wide nodule of more extensive edema and deeper erythema. In a few instances the persons complained of pain in the axilla, but general reactions did not occur or at least were not admitted.

In most instances the reactions to typhoidin were more extensive than to the control powder and in view of the difficulty frequently encountered in measuring and interpreting the reactions we have adopted the following arbitrary method of interpretation:

a. Result positive (+) when the typhoidin site alone shows a reaction or when this site has at least twice the area of erythema and greater edema as compared with the control.

b. Result doubtfully positive (\pm) when the typhoidin site is of about one-third greater intensity than the control site.

c. Result negative (—) when the reactions represent nothing more than trauma or are of about equal degree in regard to erythema and edema.

Persons tested. The cutaneous and intracutaneous typhoidin tests were made on a selected group of persons whose clinical histories were as definite as could be obtained in reference to typhoid fever in the past. The majority of these persons were selected from among medical students and laboratory assistants. All those who had been immunized had received the usual course of three injections of a vaccine freshly prepared of the army or Rawlings strain of typhoid bacillus in the usual manner.

Sera. Specimens of blood were collected aseptically and the sera used in the bactericidal tests after the method of Wright, within four hours after collection in order to utilize the native complements of each serum.

Bactericidal tests. The bactericidal power of each serum against *B. typhosus* was studied by means of the method of Wright and some of the sera were also tested by the plate method of Stern and Korte.

After extensive trials with the above and other methods we have finally adopted that of Wright as being best adapted for determining the bactericidal power of human sera under ordinary conditions. Irregular results are at times observed with the serum of the same person at different times, but it utilizes the person's own complement and lessens the influence of agglutinins which are responsible for wide errors in the plate method by reason of the fact that a clump of living bacilli may be rated and counted as one colony and thereby increase the apparent bactericidal power of a serum. In our experience this method is not adopted for measuring the bactericidal power of the sera of immunized animals because it depends upon using a volume of undiluted serum with an equal volume of culture and measuring the bactericidal power according to the number of bacilli killed and even an undiluted twenty-four hour broth culture may contain too few bacilli to exhaust the bactericidal substances in an equal volume of undiluted serum.

In this work ten dilutions of a twenty-four hour culture were used as follows: undiluted; 1:5; 1:10; 1:20; 1:40; 1:100; 1:250; 1:500; 1:5000 and 1:50,000.

In all other respects this test was conducted as described by Wright (28).

Agglutination tests. The macroscopic technic was used throughout with a culture of *B. typhosus* from Gay, also with the Rawling's strain and a strain that has been in use in agglutination work in the Bacteriological Laboratory of the Bureau of Health of Philadelphia for a number of years. Dilutions higher than 1:2500 were not employed; the results observed with these particular strains were fairly parallel.

Complement-fixation tests. Complement-fixation tests were conducted with an anti-sheep hemolytic system and two typhoid antigens prepared by suspending washed agar cultures in normal salt solution containing 0.5 per cent phenol. These antigens were titrated before the complement-fixation tests and used in amounts corresponding to one-third their anticomplementary units.

The Wassermann reactions were conducted with three different extracts, namely, an alcoholic extract of human heart re-enforced with cholesterin; an alcoholic extract of syphilitic liver and an extract of acetone insoluble lipoids of human heart.

All reactions were read immediately after the secondary period of incubation.

RESULTS

I. Bacteriolysis in vitro in relation to the anaphylactic skin reaction to typhoidin.

The results of the skin tests and a study of the sera for bactericidins, agglutinins and complement-fixing antibodies in the three groups of cases are shown in tables 1, 2 and 3.

a. Normal human sera. Normal human serum from persons who have never had typhoid fever or vaccine was found to possess a high degree of bactericidal activity over *B. typhosus* as measured by Wright's method and shown in table 1.

Not infrequently normal sera were able to kill all of the bacilli contained in an equal volume of undiluted serum during an exposure of twenty-four hours. For purposes of comparing the degree of bactericidal activity with the results of the skin and other tests, we have arbitrarily accepted as evidence of marked bacteriolysis complete sterility in all pipets and as slight bacteriolysis when growths appeared with any of the dilutions of cultures. It will be noted, however, that all sera proved bactericidal in the higher dilutions of culture as in all instances an equal volume of sera and culture diluted 1 : 50 to 1 : 50,000 in an exposure of twenty-four hours, resulted in sterility.

As shown in table 1, all of these persons showed some reaction to the intracutaneous injection of typhoidin and its control and in some instances these reactions were well marked. According to our method of interpretation the typhoidin test was positive in two persons, or 20 per cent.

As shown in table 4, which summarizes the results of the skin and bactericidal tests according to the arbitrary standard adopted, there was found no relation between the two phenomena although the sera of six of the ten persons giving negative typhoidin reactions were less active bactericidally.

b. With the sera of persons who have had typhoid fever. As shown in table 2, the majority of these persons who had had typhoid fever from one to fourteen years previously reacted positively to typhoidin and its control.

TABLE 1
Results of typhoidin skin tests and bactericidal, agglutination and complement-fixation tests among persons who had never had typhoid fever or typhoid vaccine

NO.	SKIN TEST		BACTERICIDAL TESTS ***											COMPLEMENT-FIXATION TESTS				
	Typhoidin	Control	Interpretation	1	2	3	4	5	6	7	8	9	10	11	Agglutination tests 1:20 TO 1:640	Wassermann	B. typh. (Rawlings)	B. typh. (City)
				Uncounted	Uncounted	Uncounted	Uncounted	Uncounted	20,000 to 60,000	30,000 to 120,000	600 to 2400	100 to 400	10 to 50	Control				
1	4.5 × 4.5	4.5 × 4.5	+	*	+	+	+	+	+	+	+	+	+	+	+	*	+	+
2	3.5 × 1.2	0.2 × 0.4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	0.8 × 0.8	0.8 × 0.8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	2.0 × 1.5	0.5 × 0.8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	0.5 × 0.8	0.5 × 0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	0.2 × 0.2	0.2 × 0.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	2.0 × 2.8	1.8 × 1.8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	0.6 × 0.8	1.0 × 0.6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	3.5 × 3.5	3.5 × 4.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	0.4 × 0.4	0.5 × 0.4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

* - = Complete destruction of *B. typhosus* in the bactericidal tests; absence of agglutination and negative complement-fixation tests.

† + = Growth of *B. typhosus* in the bactericidal tests.

‡ All cultures controls showed a good growth of *B. typhosus*.

TABLE 2
Results of typhoidin skin tests and bactericidal, agglutination and complement-fixation tests among persons who had typhoid fever

NO.	HISTORY	SKIN TESTS		BACTERICIDAL TESTS											AGGLUTINATION TESTS		COMPLEMENT FIXATION TESTS		
		Typhoidin	Control	Interpretation	1	2	3	4	5	6	7	8	9	10	11	I: 20 to 1: 640	Wassermann	B. typhosus (Rawlings)	B. typhosus (City)
		cm.	cm.		Uncounted	Uncounted	Uncounted	Uncounted	Uncounted	20,000 to 60,000	30,000 to 120,000	600 to 2400	100 to 400	10 to 50	Control				
1	Typhoid in 1902	1.0 × 1.2	—	+	+	+	+	+	+	+	+	+	+	+	+	1: 20	+	+	+
2	Typhoid in 1902	1.8 × 1.0	0.2 × 0.4	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+
3	Typhoid in 1904	4.0 × 4.0	4.0 × 4.0	+	+	+	+	+	+	+	+	+	+	+	+	1: 30	+	+	+
4	Typhoid in 1906	3.3 × 3.7	3.0 × 4.0	+	+	+	+	+	+	+	+	+	+	+	+	1: 20	+	+	+
5	Typhoid in 1907	2.5 × 2.0	1.5 × 0.5	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+
6	Typhoid in 1908	1.0 × 1.2	0.8 × 1.0	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+
7	Typhoid in 1908	1.0 × 1.2	0.8 × 1.0	+	+	+	+	+	+	+	+	+	+	+	+	1: 60	+	+	+
8	Typhoid in 1910	2.0 × 2.0	0.4 × 0.6	+	+	+	+	+	+	+	+	+	+	+	+	1: 80	+	+	+
9	Typhoid in 1912	2.5 × 2.5	0.4 × 0.8	+	+	+	+	+	+	+	+	+	+	+	+	1: 640	+	+	+
10	Typhoid in 1915	2.5 × 2.5	0.4 × 0.8	+	+	+	+	+	+	+	+	+	+	+	+	1: 640	+	+	+

TABLE 3
Results of typhoidin skin tests and bactericidal, agglutination and complement-fixation tests among persons who had received typhoid vaccine

NO.	HISTORY	SKIN TESTS		BACTERICIDAL TESTS											AGGLUTINATION TESTS		COMPLEMENT-FIXATION TESTS		
		Typhoidin	Control	INTERPRETATION											1:20 to 1:2500	Wassermann	B. typhosus (Rawlings)	B. typhosus (City)	
				1	2	3	4	5	6	7	8	9	10	11					
		cm.	cm.	Uncounted	Uncounted	Uncounted	Uncounted	Uncounted	20,000 to 60,000	30,000 to 120,000	600 to 2400	100 to 400	10 to 50	Control					
1	Vaccine in 1912.....	3.0 × 2.0	3.0 × 1.5	—	—	—	—	—	—	—	—	—	—	—	1:40	—	—		
2	Vaccine in 1912.....	0.6 × 0.8	0.6 × 0.6	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
3	Vaccine in 1912.....	1.2 × 1.5	0.4 × 0.6	+	+	—	—	—	—	—	—	—	—	—	1:80	—	—		
4	Vaccine in 1914.....	3.5 × 4.0	2.0 × 1.8	—	—	—	—	—	—	—	—	—	—	—	1:160	—	—		
5	Vaccine in 1914.....	4.0 × 4.0	3.5 × 4.5	—	—	—	—	—	—	—	+	—	—	—	1:120	—	+		
6	Vaccine in 1915.....	4.0 × 3.8	4.0 × 4.0	—	—	—	—	—	—	—	—	—	—	—	1:80	—	—		
7	Vaccine in 1915.....	0.6 × 1.0	0.2 × 0.4	+	+	—	—	—	—	—	—	—	—	—	1:640	—	—		
8	Vaccine 6 mos. ago..	2.0 × 1.5	0.8 × 1.0	—	—	—	—	—	—	—	—	—	—	—	1:640	—	—		
9	Vaccine 5 mos. ago..	1.6 × 1.8	2.2 × 2.2	—	—	—	—	—	—	—	—	—	—	—	1:640	—	—		
10	Vaccine 5 mos. ago..	4.0 × 4.0	4.0 × 4.0	—	—	—	—	—	—	—	—	—	—	—	1:640	—	—		
11	Vaccine 4 mos. ago..	2.5 × 3.0	1.0 × 1.5	—	—	—	—	—	—	—	—	—	—	—	1:640	+	+		
12	Vaccine 3 mos. ago..	4.0 × 4.0	2.0 × 2.0	+	+	—	—	—	—	—	—	—	—	—	1:160	+	+		
13	Vaccine 1 mo. ago...	1.0 × 0.8	—	—	—	—	—	—	—	—	—	—	—	—	1:2500	—	—		
14	Vaccine 1 mo. ago...	1.0 × 0.8	—	—	—	—	—	—	—	—	—	—	—	—	1:1280	—	—		
15	Vaccine 1 mo. ago...	1.0 × 1.5	0.4 × 0.6	+	+	—	—	—	—	+	—	—	—	—	1:640	+	+		
16	Vaccine 1 mo. ago...	1.0 × 1.5	0.4 × 0.6	+	+	—	—	—	—	—	—	—	—	—	1:2500	+	+		
17	Vaccine 1 week ago.	1.0 × 1.0	0.2 × 0.6	+	+	—	—	—	—	—	—	—	—	—	1:2500	+	+		
18	Vaccine 1 week ago.	2.0 × 4.5	1.0 × 2.0	+	+	—	—	—	—	—	—	—	—	—	1:2500	+	+		
19	Vaccine 1 dose.....	0.8 × 1.0	0.2 × 0.4	+	+	—	—	—	—	—	—	—	—	—	1:140	—	—		
20	Vaccine 2 doses.....	1.0 × 1.5	0.6 × 0.8	+	+	—	—	—	—	—	—	—	—	—	1:80	+	+		
21	Vaccine 2 doses.....	1.0 × 1.8	0.2 × 0.4	+	—	—	—	—	—	—	—	—	—	—	1:160	—	—		
22	Vaccine 2 doses.....	1.0 × 1.8	0.2 × 0.4	+	—	—	—	—	—	—	—	—	—	—	1:160	—	—		

* All culture controls showed good growths.

TABLE 4

Relation of bacteriolysin (in vitro) and typhoidin skin reaction among normal persons

TOTAL	MARKED BACTERICIDAL AND NEGATIVE SKIN TEST	MARKED BACTERICIDAL AND POSITIVE SKIN TEST	SLIGHT BACTERICIDAL AND NEGATIVE SKIN TEST	SLIGHT BACTERICIDAL AND POSITIVE SKIN TEST
10	2	1	6	1

In six or 60 per cent the typhoidin reactions were regarded as positive; the relation of the bactericidal power of the serum to the results of the skin tests according to our arbitrary interpretation is shown in table 5:

TABLE 5

Relation of Bacteriolysis (in vitro) and typhoidin skin reactions among persons who have had typhoid fever

NO.	MARKED BACTERICIDAL AND NEGATIVE SKIN TEST	MARKED BACTERICIDAL AND POSITIVE SKIN TEST	SLIGHT BACTERICIDAL AND NEGATIVE SKIN TEST	SLIGHT BACTERICIDAL AND POSITIVE SKIN TEST
10	3	4	1	2

While the bactericidal activity of the sera of these persons and the percentage of positive skin reactions were in general higher than observed among normal persons, a high bactericidal power of the serum and a positive skin reaction occurred together in but 40 per cent of this small series.

c. With the sera of persons who have had typhoid vaccine. The results observed among twenty-two persons who had received the full course of vaccine injections at various times before these tests were made is shown in table 3.

In 13 or about 60 per cent the typhoidin tests were regarded as positive; the relation of the bactericidal activity of the sera to the skin reactions are summarized in table 6:

TABLE 6

Relation of bacteriolysis (in vitro) and typhoidin skin reactions among persons who have had typhoid vaccine

NO.	MARKED BACTERICIDAL AND NEGATIVE SKIN TEST	MARKED BACTERICIDAL AND POSITIVE SKIN TEST	SLIGHT BACTERICIDAL AND NEGATIVE SKIN TEST	SLIGHT BACTERICIDAL AND POSITIVE SKIN TEST
22	4	8	5	5

While immunization may be said to have increased the bactericidal power of the sera of some of these persons and certainly resulted in sensitizing a number to typhoid protein, there was found no constant relation between the results of the skin tests and the bactericidal power of the sera.

Bacteriolysis in vitro by the plate method. A number of sera were tested in dilutions ranging from 1 : 100 to 1 : 204,800 by the method of Stern and Korte with a 1 : 200 dilution of a 24 hour broth culture, but the results were quite irregular. The sera of a number of guinea-pigs used as complement was found in a dose of 0.5 cc. of a 1 : 10 dilution to possess some bactericidal power over *B. typhosus* and thereby introduced an element of error. Our controls were uniformly satisfactory and culture controls showed innumerable colonies, but the difference in counts was not uniform. Not frequently and particularly with the sera of persons recently immunized, a few colonies were found in a whole series of plates containing the full series of dilutions and while showing thereby evidences of a bactericidal power of the serum even in the 1 : 204,800 dilution, the graduations in bactericidal activity according to the dilution of serum were not even approximately in evidence. While it is not possible to correlate closely the results of this method and that of Wright, because the former employs a constant dose of culture with dilutions of serum and the complement of an alien animal and the latter a constant dose of undiluted serum, native complement and dilutions of culture, our results show that in general the bactericidal action of the same serum was in evidence or absent in both methods.

II. The relation of agglutination to the typhoidin skin reaction.

The results shown in tables 1, 2 and 3 may be summarized as follows:

1. Of the serum of ten normal persons two gave partial agglutination in dilution of 1 : 20; one of these persons gave a positive skin reaction while the second did not (table 1).
2. Of the sera of ten persons that had had typhoid fever one to fourteen years previously, six gave agglutination in dilutions

ranging from 1 : 20 to 1 : 640 (table 2); of these four gave positive skin reactions while two did not.

3. Of the sera of twenty-two persons that had received typhoid vaccine at various times preceding these tests, 21 gave agglutination in dilutions varying from 1 : 40 to 1 : 2500 (higher dilutions not employed); of these, 13 gave positive skin reactions and 9 did not.

In our experience agglutinins are present in the blood serum of the majority of persons reacting positively in the skin test, but there is no definite relation between the two as either may be in evidence in the absence of the other.

Agglutinins were most in evidence in the sera of immunized persons (table 3), and particularly among those that had been immunized within a year of the time these tests were made. After two or three years the agglutinin titer fell and likewise the percentage of positive typhoidin reactions.

III. The relation of complement-fixation to the typhoidin skin reaction.

The results shown in tables 1, 2 and 3 in this connection may be summarized as follows:

1. The sera of ten normal persons did not absorb complement with typhoid antigen; two of these persons gave a positive typhoidin reaction (table 1).

2. Of the sera of ten persons that had had typhoid fever four gave weak complement-fixation with one or both typhoid antigens; two of these persons gave positive skin reactions while two did not (table 2).

3. Of the sera of twenty-two persons that had received typhoid vaccine, ten gave positive complement-fixation reactions with one or both typhoid antigens; seven of these persons gave positive skin tests while three did not (table 3).

The majority of the complement-fixation reactions, and particularly the stronger of these reactions, occurred with the sera of immunized persons and especially those that had received vaccine within a year of the time when these tests were made.

According to our experience the majority of complement-fixation reactions occurred with the sera of those persons that gave positive typhoidin reactions and particularly among the immunized individuals, but there is no definite relation between the two phenomena as skin reactions may occur among persons whose sera do not absorb complement with typhoid antigen and vice versa. Probably if a polyvalent typhoid antigen had been used as suggested by Garbat and others a higher percentage of positive complement-fixation reactions might have resulted, but it was our purpose to work with the same monovalent antigen as was used in the agglutination tests.

Agglutinins are present or, at least, are detected in a larger percentage of sera than are complement-fixing antibodies. In this series of 42 persons agglutinins were present (dilutions 1:20 and higher) in about 70 per cent and complement-fixing antibodies in about 33 per cent.

THE TYPHOIDIN SKIN REACTION

While our series of tests with powder typhoidin and its control is too small to draw any conclusions, our results and experience may be summarized as follows:

1. Both are quite irritant when injected intracutaneously in amounts of 0.001 and 0.0005 mgm. The smaller of these doses of both typhoidin and the control powder produced reactions in normal persons, but the reactions were less severe.

2. It is not clear what causes these non-specific reactions, but it is suggested that substances from the culture media may be responsible comparably with the pseudoreactions that may follow the intracutaneous injection of plain broth or that used in the preparation of the toxin for the Schick test. It is also probable that soluble toxic substances of typhoid bacilli in the nature of endotoxins, may be responsible and that a purer typhoidin, freed as far as possible of toxic substances and composed of the typhoid protein alone, may yield sharper and more specific results. One of us (Kolmer) with Dr. Matsunami is endeavoring at the present time to produce an anaphylactogen of this character.

3. While we naturally hesitate to discuss the reactions in view of the marked irritant or non-specific reactions following the injection of the control powder, our results may be given as follows, as based upon the method of interpretation mentioned in the earlier part of the paper:

TABLE 7
Results of typhoidin skin tests

HISTORY	TOTAL	POSITIVE REACTIONS	NEGATIVE REACTIONS
Normal.....	10	2	8
Typhoid 1 to 8 years ago.....	5	4	1
Typhoid 8 to 14 years ago.....	5	2	3
Vaccine within 1 year.....	15	10	5
Vaccine 1 to 4 years ago.....	7	2	5

Among the ten persons that claim never to have had the fever and that are known not to have received vaccine, the two persons giving positive skin reactions may, of course, have had an unrecognized attack of the fever, but this is not probable. Hypersensitiveness is apparently more lasting after typhoid fever than after active immunization with a vaccine; in our experience cutaneous anaphylaxis rapidly diminishes after the first year following immunization.

DISCUSSION

In accepting the evidence of cutaneous anaphylaxis as an index of defensive activity or immunity to bacterial infection, two theories regarding the mechanism of the immunity may be entertained.

First, the cutaneous anaphylactic reaction may be taken as an index that the body cells have been brought in contact with a certain bacterial protein and that in addition to the production of the anaphylactic state, antibodies of a protective and curative nature are produced; furthermore that as long as the anaphylactic state is demonstrable these antibodies may be assumed to be present in the cells and body fluids. According to this theory the anaphylactic antibody may be entirely within the

cells and the reaction cellular, while the protective antibodies may be either within the cells, in the body fluids or both.

Secondly, the same mechanism and antibodies responsible for the cutaneous anaphylactic reaction may be capable of attacking and destroying the living microörganism and represent therefore true antibodies. This theory would correspond more closely with the humoral or chemical theory of anaphylaxis which is being attacked by an increasing number of investigators.

Personally we subscribe to the theory first mentioned and look upon the typhoidin and other cutaneous anaphylactic reactions as indicating in themselves nothing more than sensitization to a particular complex protein; at the same time this condition of cutaneous anaphylaxis may be co-incident with the presence of protective and curative antibodies or a condition of the body cells prepared to furnish these upon stimulation through the presence of the living microörganism or its products. In this manner the anaphylactic reaction and the typhoidin reaction in particular, may be regarded as an indicator of immunity in the same manner as investigators for years have looked upon the typhoid agglutinin, but our own investigations indicate that cutaneous anaphylaxis cannot be accepted as direct or reliable evidence of actual immunity any more than the agglutinins in the same capacity.

While the role of agglutinin and bacteriolysin in typhoid immunity has been minimized, yet it is generally agreed and supported by our own experiments, that both are present in the body fluids in the majority of persons during and for some time after typhoid fever and after immunization. Likewise our experiments show that these antibodies and particularly the agglutinin, are especially likely to be present in the sera of those persons showing typhoid hypersensitiveness, and while there is no close relation between the skin reaction and the bactericidal substances as studied *in vitro*, this may be due to faulty technic rather than actual conditions.

Sufficient time has not yet elapsed to judge of the ultimate value of typhoid immunization and of the skin or agglutination test as an index of immunity. While animal tests have proven

successful in the hands of Professor Gay and others in determining the relative value of typhoid vaccines (29), we have not so far been able to obtain definite results bearing upon the subject at hand. Immunized animals, which do not give a skin reaction show considerable protection against the intravenous injection of a pathogenic strain, but their sera likewise shows a high agglutinin and bacteriolys in titer; at present we are endeavoring to immunize rabbits sufficiently to yield a skin reaction and test their protection and agglutinin and bacteriolysin content at varying times after cutaneous anaphylaxis has disappeared. In this manner more definite information may be obtained of the value of cutaneous anaphylaxis as an indicator of immunity.

SUMMARY

1. While the bactericidal power of human sera over *B. typhosus* is increased in a proportion of persons following typhoid fever or active immunization with a vaccine, there is no direct relation between the typhoidin skin reaction and the results of bactericidal tests *in vitro*.

2. Agglutinins or complement-fixing antibodies or both are present in the blood serum of the majority of persons reacting positively in the skin test and particularly among those actively immunized, but there is no definite relation between these as either may be in evidence in the absence of the other.

3. Powdered typhoidin and its control produces severe reactions when injected intracutaneously in doses of 0.0005 to 0.001 mgm.; these reactions and particularly that produced by the control, render the reading and interpretation of the test quite difficult and subject to much error.

4. Cutaneous anaphylaxis to typhoidin was found apparently to persist for a longer time among those who have had typhoid fever than among those actively immunized with the vaccine. Among the latter the highest percentage of reactions was found during the first year following immunization.

5. While the typhoidin reaction indicates sensitization to typhoid protein, there is not yet sufficient evidence to warrant its acceptance as an index of immunity in typhoid fever.

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THE RELATION OF THE LUETIN SKIN REACTION TO IMMUNITY IN SYPHILIS (INCLUDING THE TREPONEMICIDAL ACTION OF HUMAN SERUM IN SYPHILIS)¹

ANAPHYLACTIC SKIN REACTIONS IN RELATION TO IMMUNITY. II

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Although numerous investigations have been made on the subject of antibodies and immunity in syphilis, our knowledge is quite imperfect and incomplete. It is the opinion of Neisser and Bruck (1) and others that true immunity in syphilis has not been demonstrated and probably does not exist. However, with the isolation of *treponema pallidum* in pure culture by Noguchi more accurate studies were possible, and agglutinins for cultures of *pallida* have been demonstrated by Kolmer (2) in the sera of immunized rabbits and by Nakano (3), Kissmeyer (4), Zinsser and Hopkins (5) and Kolmer, Broadwell and Matsunami (6) in the sera of immunized animals and human syphilitics. The first syphilis antibody discovered was that concerned in the absorption of complement with lipoidal tissue extracts by Wassermann and Bruck and Detre in 1905 and by Noguchi in 1912 with antigens composed of pure cultures of *treponema pallida*.

On the basis of work done prior to 1911 with emulsions of tissues rich in *pallida*, Neisser and Bruck expressed the opinion that parasitocidal antibodies do not occur in the course of syphilis; recently Zinsser and Hopkins (7) have shown that the sera of

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rabbits and sheep immunized with a pure culture of *pallida* developed spirocheticidal antibodies for culture *pallida*, but later Zinsser, Hopkins and McBurney (8) showed that these antibodies had practically no action for virulent treponemata obtained directly from lesions.

From the standpoint of lytic immunity in syphilis, the determination of the presence or absence of treponemicidal substances in the body fluids in this disease is of considerable importance, and this constitutes the chief object of the present study as based upon treponemicidal tests *in vitro* with a culture of *pallida*.

As part of a series of studies bearing upon the question of anaphylactic skin reactions in relation to immunity, the luetin test was performed on a large series of persons, mainly those in the tertiary and latent stages of syphilis and showing involvement of the tissues of the central nervous system. The sera of a number of these persons yielding typical luetin reactions of varying degrees of severity; also the sera of syphilitic persons yielding negative luetin reactions and the sera of normal non-syphilitic persons were tested for treponemicidal activity *in vitro* and also for agglutinin and complement-fixing power, in order to determine the relation, if any, between the occurrence of the anaphylactic skin reaction and the presence or absence of these antibodies.

METHOD

Luetin tests. These were conducted with Noguchi's luetin so diluted with sterile normal salt solution that 0.1 cc. contained the dose indicated. The reactions were read at the end of forty-eight hours after injection and watched for some time longer for later developments. As pointed out recently by Sherrick (9), normal persons taking potassium iodide may yield reactions to the intracutaneous injection of luetin. We have been able to confirm this discovery, the results of our investigation being given in another communication (10), but here we wish to state that none of our patients nor the normal persons used as controls were taking iodides at or near the time of these tests.

Only those persons were selected for further study who gave well marked positive or definitely negative reactions.

Blood was collected aseptically and in the treponemicidal tests the

sera were used within sixteen hours in order to utilize the patient's own complements.

*Persons studied.*² Normal non-syphilitic persons were selected from among laboratory assistants and medical students; for syphilitic persons we have mainly selected those in the late stages of the disease and particularly those suffering with clinically well defined paresis, because this disease has been so definitely established as due to syphilis by Noguchi and Moore, Wile and others, living spirochetes being obtained from the brain tissue, and the blood and cerebrospinal fluid Wassermann reactions being strongly positive in practically all cases. In other words, this group of cases seemed to us best suited for these treponemicidal studies, particularly so since other antibodies as pallida agglutinin and Wassermann antibody are present in well marked degree and many cases yield positive luetin skin reactions.

Culture. A considerable part of the work and all of that reported herein, was conducted with Dr. Zinsser's strain A of pallida cultivated anerobically in ascites broth over slants of Dorsett's egg medium. After three to four weeks good growths were secured, which, after removal, brief shaking and brief centrifugalization were diluted with sufficient sterile salt solution until from eight to twelve active treponemata were found to each field as examined by dark field illumination. This number of treponemata has been found by Zinsser and Hopkins not so large as to obscure the possibility of a positive result, in that incomplete killing could not be differentiated from absolute failure to kill, while furnishing a sufficient number to render the results definite. In our experience the number used fulfills the object of delicacy and particularly so under the conditions of the treponemicidal tests as we have been required to repeat the work with sera when the number of treponemata used were less and too few to give uniform growths in the controls.

Treponemicidal tests. We have practically followed the technic employed by Zinsser and Hopkins with rabbit immune serum; all sera were used within sixteen hours after collection, being kept on ice from the time of bleeding until these tests were set up, in order to utilize the person's own complements. Each serum was tested in duplicate as follows: Into a series of sterile test tubes varying amounts of serum

² We are greatly indebted to Dr. J. Allen Jackson and Dr. Immerman, chief resident and assistant resident physicians respectively in the Department for the Insane, Philadelphia General Hospital, for their coöperation and the clinical facilities afforded us in this work.

and pallida culture were mixed and the total volume brought up to 1.0 cc. with the addition of sterile normal salt solution:

Tube 1. 0.1 cc. serum and 0.1 cc. culture.

Tube 2. same as No. 1 (duplicate).

Tube 3. 0.2 cc. serum and 0.2 cc. culture.

Tube 4. same as No. 2 (duplicate).

Tube 5. 1.0 cc. serum and 0.5 cc. culture.

Tube 6. same as No. 5 (duplicate).

In the first four tubes of each set equal parts of serum and culture were used; in the remaining two tubes the amount of serum was double that of the culture (salt solution omitted).

In all experiments from six to twelve culture controls, 0.1, 0.2 and 0.5 cc. culture being used with sufficient sterile salt solution to make the total volume 1.0 cc., were included.

All tubes were gently shaken and incubated at 37° C. for 2 to 3 hours, when a small piece of sterile rabbit kidney and 8 cc. of ascites-agar were added to each tube followed by a layer of sterile paraffin oil.

After eighteen to twenty-four days incubation and when all of the controls showed good growths of pallida, all tubes were examined with dark field illumination. In case the controls were sterile or irregular the results were excluded and the work repeated.

We have made no attempt to judge a diminution in the number of treponemata, as the number found in a preparation as examined by dark field illumination will vary in the same tube according to the level from which the preparation is made.

Agglutination tests. The macroscopic technic was employed as described in a previous communication (6) with ascites broth cultures of the same culture of pallida and in dilutions varying from 1 : 2 to 1 : 80; the results were read after 2 hours' incubation and standing in a refrigerator over night and always with the aid of dark field illumination.

Complement-fixation tests. Each serum was submitted to the Wassermann reaction with three lipoidal extracts, namely, an alcoholic extract of human heart re-enforced with cholesterin (C. H.), an alcoholic extract of syphilitic liver (S) and an extract of acetone-insoluble lipoids of human heart (A). Luetin was also used as an antigen after preliminary titration and in dose equivalent to one-third the anticomplementary unit.

In all tests the results were read and recorded immediately after the second period of incubation.

RESULTS

I. Treponemolysis by human sera in vitro in relation to the anaphylactic skin reaction to luetin

Treponemicidal, agglutinating and complement-fixing properties of normal human serum. The results observed with the sera of seven healthy non-syphilitic adults are shown in table 1.

TABLE 1

Treponemicidal properties of normal human serum; also agglutinating and complement-fixing properties

NO.	LUETIN SKIN TEST	TREPONEMICIDAL TESTS*	AGGLUTINATION TESTS†						COMPLEMENT- FIXATION TESTS			
			1:2	1:5	1:10	1:20	1:40	1:80	Lue- tin	C. H.	S	A
1	Negative	Good growths	—	—	—	—	—	—	—	—	—	—
2	Negative	Good growths	±	—	—	—	—	—	—	—	—	—
3	Negative	Good growths	—	—	—	—	—	—	—	—	—	—
4	Negative	Good growths	±	—	—	—	—	—	—	—	—	—
5	Negative	Good growths	±	—	—	—	—	—	—	—	—	—
6	Negative	Good growths	—	—	—	—	—	—	—	—	—	—
7	Negative	Good growths	—	—	—	—	—	—	—	—	—	—

* All controls showed good growths; all tubes including duplicates in the treponemicidal tests showed good growths that were equal to the controls.

† — = no evidences of agglutination; ± = partial agglutination; + = well marked agglutination.

In most instances the luetin skin tests produced a very small nodule and area of erythema, which had largely disappeared in forty eight hours and were regarded as negative reactions.

With each serum there were no evidences of treponemicidal activity; the partial agglutination of pallida in the lowest dilution of serum (equal parts of serum and culture) corresponds exactly with the results of our previous work on the agglutination of this strain of culture pallida by the sera of normal persons (6). In all instances the complement fixation tests were negative with all antigens including the delicate cholesterinized extract.

Treponemicidal, agglutinating and complement-fixing properties of the sera of paretics in comparison with the anaphylactic skin

reactions to luetin. As previously stated, these patients were selected particularly in this investigation because paresis has been so firmly established as syphilitic and as the concentration of antibodies is most likely to be found in the body fluids in this disease. The results observed with the sera of twenty-three paretics are shown in table 2.

TABLE 2

Treponemicidal properties of human serum in paresis; comparative results of the luetin, treponemicidal, agglutination and complement-fixation tests

NO.	LUETIN SKIN TEST*	TREPONEMICIDAL TESTS	AGGLUTINATION TESTS						COMPLEMENT-FIXATION TESTS†			
			1:2	1:5	1:10	1:20	1:40	1:80	Leu-tin	C. H.	S	A
1	—	Good growths	+	+	±	—	—	—	—	+	—	—
2	—	Good growths	+	+	+	+	+	—	+	++++	++++	++++
3	+	Good growths	+	+	+	—	—	—	—	++++	++++	++++
4	—	Good growths	+	+	±	—	—	—	—	++++	++++	++++
5	+	Good growths	+	+	±	—	—	—	—	++++	++++	++++
6	+	Good growths	+	+	+	+	+	—	+	++++	++++	++++
7	—	Good growths	+	±	—	—	—	—	+	++++	++++	++++
8	+	Good growths	—	—	—	—	—	—	—	++	++	++
9	++	Good growths	+	+	+	—	—	—	—	+++	+++	+++
10	++	Good growths	+	+	+	—	—	—	—	++++	++++	++++
11	—	Good growths	+	+	+	+	—	—	—	±	—	—
12	—	Good growths	+	+	—	—	—	—	+	++++	++++	++++
13	—	Good growths	+	+	—	—	—	—	—	+	—	—
14	—	Good growths	+	+	+	+	+	+	+	++++	++++	++++
15	+	Good growths	+	+	+	+	+	—	—	+	+	+
16	+	Good growths	+	+	+	+	+	—	+	++++	—	—
17	++	Good growths	+	+	+	+	—	—	—	++++	++++	++++
18	—	Good growths	+	+	+	+	+	—	—	++++	+	++
19	—	Good growths	+	+	+	—	—	—	—	++++	++++	++++
20	+	Good growths	+	+	—	—	—	—	±	++++	++++	++++
21	++	Good growths	+	+	+	—	—	—	±	++++	±	++++
22	++	Good growths	+	—	—	—	—	—	—	++++	+	++++
23	+	Good growths	+	+	+	+	—	—	—	++++	—	++++

* In the luetin tests + = papular reaction; ++ = severe papular or pustular reaction; — = negative reaction.

† +++++ = complete inhibition of hemolysis (strongly positive).

+++ = 75 per cent inhibition of hemolysis (moderately positive).

++ = 50 per cent inhibition of hemolysis (weakly positive).

+ = 25 per cent inhibition of hemolysis (weakly positive).

An examination of this table shows the following:

1. In no instance were there any appreciable evidences of a treponemicidal action of these sera. In every case and in each tube including the duplicates, good rich growths of treponemata were found comparable in numbers and vitality and in evidences of proliferation to the treponemata in the numerous controls.

2. About 56 per cent of these patients yielded positive luetin reactions of varying degree as indicated, but the sera of all, irrespective of the results of the anaphylactic skin tests, failed to show any appreciable lytic effect upon the culture of pallida employed.

3. If agglutination in dilutions of 1 : 5 and higher is regarded as evidence of the presence of immune agglutinin for this particular culture of pallida, the sera of all but two patients or 91 per cent showed the presence of an appreciable amount of agglutinin, the extent of agglutination being shown in the table.

4. The sera of all reacted positively in the Wassermann reaction, although three sera were positive with the cholesterinized extract alone, which demonstrates the superior antigenic sensitiveness of these extracts. With 9 sera or about 40 per cent, weak complement-fixation was observed with the antigen of luetin.

Treponemicidal, agglutinating and complement-fixing properties of sera in tertiary and latent syphilis in comparison with the anaphylactic skin reactions to luetin. The results observed with the sera of twelve persons of whom the clinical history, laboratory tests or luetin reaction indicated tertiary or latent syphilis, mainly involving the tissues of the central nervous system, are shown in table 3.

Among these cases the percentage of positive luetin reactions was higher than shown in table 2, but in no instance were there any appreciable evidences of a treponemicidal activity on the part of the sera.

The sera of the majority of these patients showed the presence of appreciable amounts of agglutinin for pallida culture as shown in the table and yielded positive Wassermann reactions particularly with the cholesterinized extract.

TABLE 3

Treponemicidal properties of human serum in tertiary syphilis; comparative results of the luetin, treponemicidal, agglutination and complement-fixation tests

NO.	DIAGNOSIS	LUE- TIN SKIN TEST	TREPONEMICIDAL TESTS	AGGLUTINATION TESTS						COMPLEMENT-FIXATION TESTS			
				1:2	1:5	1:10	1:20	1:40	1:80	Lue- tin	C. H.	S	A
1	Tabo-paresis	+	Good growth	+	-	-	-	-	-	+	++++	++++	++++
2	Tabo-paresis	-	Good growth	+	+	+	-	-	-	-	++++	++++	++++
3	Tabes dorsalis	++	Good growth	+	+	+	+	-	-	-	+	-	-
4	Cerebrospinal syphilis.....	++	Good growth	+	+	+	+	-	-	++	++++	++++	++++
5	Cerebrospinal syphilis.....	±	Good growth	+	-	-	-	-	-	-	++++	++++	++++
6	Depressive mania.....	+	Good growth	+	+	+	+	-	-	-	+	-	-
7	Dementia pre- cox.....	++	Good growth	+	+	±	-	-	-	-	-	-	-
8	Dementia pre- cox.....	-	Good growth	+	+	+	-	-	-	-	-	-	-
9	Dementia pre- cox.....	+	Good growth	+	+	-	-	-	-	-	-	-	-
10	Dementia pre- cox.....	++	Good growth	+	+	+	+	+	+	-	-	-	-
11	Dementia pre- cox.....	+	Good growth	+	+	-	-	-	-	-	+	-	-
12	Aortic disease	++	Good growth	+	+	+	-	-	-	+	++++	++++	++++

Additional experiments with the sera of 14 persons included in tables 2 and 3, yielding positive Wassermann reactions and positive and negative skin reactions and with the sera of 11 normal non-syphilitic persons were conducted by mixing in a series of small sterile test tubes equal parts of fresh active serum and young ascites-broth culture of pallida, keeping the mixture at 37°C. and examining it every half hour with dark field illumination for evidences of agglutination and treponemolysis. At the end of two and one-half hours four of the sera from syphilitic persons had caused slight agglutination, but no other effect upon the treponemas could be detected; at the end of twenty-four hours ten of the syphilitic and two of the normal sera had produced agglutination indicating that agglutination of pallida occurs slowly; but some motile treponemata were found and there were no direct evidences of treponemolysis.

II. Agglutination of culture pallida in relation to the anaphylactic skin reaction to luetin

That the occurrence of the skin reaction in syphilis to luetin cannot be taken as an indication that appreciable and demonstrable amounts of pallida antibodies are present in the body fluids is indicated in the summary of tables 2 and 3 given in table 4 regarding the presence of one antibody, namely, agglutinin for culture pallida, in relation to the results of the skin test. In compiling this table, agglutination was regarded as positive when occurring in dilutions of 1 : 5 and higher.

TABLE 4

Agglutination of treponema pallida in relation to the luetin skin reaction

TOTAL	+ LUETIN AND + AGGLUTININ	- LUETIN AND + AGGLUTININ	+ LUETIN AND - AGGLUTININ
35	19 or 54 per cent	12 or 34 per cent	4 or 11 per cent

While the sera of all of the normal non-syphilitic persons yielding negative luetin reactions (table 1) failed to agglutinate the culture of pallida in dilutions of 1 : 5 or higher, the sera of only 50 to 60 per cent of syphilitics yielding positive skin reactions showed appreciable amounts of agglutinin; furthermore positive skin reactions occurred among persons whose sera did not contain appreciable amounts of agglutinin for this strain of pallida.

III. Complement fixation in relation to the anaphylactic skin reaction to luetin

It is well known that the Wassermann and luetin reactions do not run parallel; in general the Wassermann reaction is positive in a larger percentage of syphilitic persons than the luetin skin reaction. In our series of 23 paretics the Wassermann reaction as based upon the results observed with the three antigens and particularly the cholesterinized extract, was positive in all or 100 per cent; the luetin reaction was positive in 13 or 56 per cent. It has been clearly established, therefore, as previously

stated, that there is no definite relation between hypersensitivity to pallida protein and the presence of the Wassermann antibody in the body fluids although comparative studies show that the sera of the majority of persons reacting positively to the luetin skin test, yield positive Wassermann reactions.

Of more interest in this connection is the relation between complement fixation with pallida antigen (luetin) and the luetin skin reaction in view of the claims that both may be regarded as an index of defensive activity. We have summarized the results shown in tables 2 and 3 in table 5.

TABLE 5

Complement fixation with luetin antigen in relation to the luetin skin reaction

TOTAL	+ COMPLEMENT FIXATION AND + SKIN REACTION	- COMPLEMENT FIXATION AND + SKIN REACTION	+ COMPLEMENT FIXATION AND - SKIN REACTION	- COMPLEMENT FIXATION AND - SKIN REACTION
35	9 or 26 per cent	14 or 40 per cent	3 or 9 per cent	9 or 26 per cent

It is the consensus of opinion at present that the Wassermann reaction is not a true antigen-antibody reaction, and Noguchi has regarded complement fixation with pure cultures of pallida as a specific antigen-antibody reaction due to a specific pallida antibody and an index of defensive activity (11). If this is true, the results of our study indicate that the presence of this specific amboceptor-like antibody and pallida hypersensitiveness occurred together in but 26 per cent of our cases; both were absent in 26 per cent and hypersensitiveness was present while the amboceptor was absent in about 40 per cent. In all instances complement fixation with luetin an antigen occurred only with the sera of those persons yielding positive Wassermann reactions with the usual tissue or lipoidal extracts and we have previously expressed the opinion on the basis of experiments (12) that complement fixation with treponema antigen is probably due to the same substance or antibody as is concerned in the Wassermann reaction and dependent upon the presence of lipoidal substances in the pallida antigen. The results of these comparative tests indicate that there is no direct relation between hyper-

sensitiveness and complement fixation; both reactions indicate an infection with *treponema pallida*, but neither can be regarded as indicating the co-existence of a *treponemolysin* or as an index of actual lytic immunity as based at least upon the results of *treponemicidal* tests *in vitro*.

Of further interest in this connection is the relation found between the occurrence of agglutination of culture *pallida* and complement fixation with luetin as antigen and particularly on the basis that complement fixation with this antigen is due to a specific antibody separate from that concerned in the Wassermann reaction. The results are shown in table 6.

TABLE 6
Agglutination of culture pallida and complement fixation with luetin

TOTAL	+ AGGLUTINATION AND + COMPLEMENT FIXATION	+ AGGLUTINATION AND - COMPLEMENT FIXATION	- AGGLUTINATION AND - COMPLEMENT FIXATION	- AGGLUTINATION AND + COMPLEMENT FIXATION
35	9 or 26 per cent	22 or 63 per cent	2 or 5 per cent	2 or 5 per cent

Our results show that agglutination of culture *pallida* occurs with a higher percentage of sera (regarding agglutination in dilutions of 1 : 5 and higher as positive) than complement fixation with luetin as antigen; in only 26 per cent of sera were both *pallida* agglutinin and complement fixing antibody (amboceptor) found together. In our opinion these differences are largely due to the poor antigenic qualities of luetin in the complement fixation test and a better idea regarding the occurrence of both agglutinin and complement fixing antibody in the sera of syphilitics is gained by conducting the latter test with the usual lipoidal tissue extracts; under these conditions we observed that both agglutination and complement fixation occurred with about 75 per cent of sera.

IV. Protection in relation to the anaphylactic skin reaction to luetin

In a strict sense the question whether or not an anaphylactic skin reaction may be taken as an index of defensive activity can only be answered on the basis of actual protection experiments.

In reference to the luetin skin reaction this may be studied experimentally by injecting animals (rabbits and monkeys) with pallida cultures until a skin reaction can be elicited and then inoculating with virulent pallida to determine if infection will result. Experiments of this nature have been done by Dr. Noguchi (personal communication), the results of which we are able to mention here with his permission. Twelve rabbits were immunized with a culture of pallida over a period of five months; after giving a rest of one month the luetin skin test was conducted with definite reactions in all, some becoming pustular after the fifth day. These animals were afterwards tested by inoculating into the testicles with a virulent pallida directly obtained from the syphilitic testicles of rabbits. Six of these took on both sides, while the others either showed one-sided orchitis or remained uninfected. It is Dr. Noguchi's impression that the immunization reduced susceptibility in some rabbits, while it had no effect whatsoever in others; in fact he found that among those animals which took the inoculation, some developed a generalized syphilis—a phenomenon which in his experience hardly ever is observed in rabbits with the strains with which he was working.

Numerous attempts, particularly by Neisser and Bruck (1) and Nakano (3) to immunize animals against syphilis have failed; Metchnikoff (13) claims to have succeeded by inoculating with syphilis directly, but Neisser disputes the experiments and advances the claim that the syphilitic is not immune to syphilis, but may be re-infected and develop the later manifestations of the disease without showing a discernible primary lesion. Unfortunately the skin reactions were not employed in their work but it is reasonable to assume that at least some of the animals used in Neisser's and Nakano's experiments were injected sufficiently to sensitize against pallida protein and that a skin reaction could have been elicited. Furthermore it is doubtful whether spontaneous cure ever occurs in human syphilis, that is, entirely unaided by drugs or treatment of any kind; many persons yielding positive anaphylactic skin reactions show exacerbations and continued progression of their infection until death results, so

that evidence of this nature and experiments tend to indicate that a state of anaphylaxis to a particular protein may exist without actual or at least effectual resistance to the corresponding micro-parasite.

SUMMARY

The results of this study conducted with one culture of *treponema pallidum* may be summarized as follows:

1. Fresh active normal serum exerts no treponemicidal action on culture pallida *in vitro* in the proportion of one or two parts of serum to one part of diluted fluid culture.

2. The sera of persons in the tertiary and latent stages of syphilis exert no appreciable treponemicidal action on culture pallida *in vitro* under similar conditions.

3. The sera of syphilitic persons yielding typical luetin reactions did not exert any treponemicidal activity *in vitro*.

4. There is no direct relation between the occurrence of cutaneous hypersensitiveness to luetin and the presence of agglutinin for culture pallida in the blood serum.

5. There is no direct relation between the occurrence of cutaneous hypersensitiveness to luetin and the presence of a complement-fixing antibody with an antigen of luetin in the blood serum; both the anaphylactic and complement-fixing antibodies were present or absent together in 52 per cent of cases, while the former was present and the latter absent in 40 per cent. The anaphylactic and complement-fixation reactions indicate an infection with *treponema pallidum*, but as based upon experiments *in vitro* neither can be regarded as indicating the co-existence of a treponemolysin or as an index of immunity to syphilis.

6. The sera of a larger percentage of syphilitics contain agglutinin for *treponema pallidum* than complement-fixing antibody when luetin is used as antigen. It is probable that this difference is due in large part to the poor antigenic sensitiveness of luetin as antigen; when ordinary lipoidal extracts were used as antigens both complement fixation and agglutination occurred together with the majority of sera.

7. As based mainly upon the results of these treponemicidal

and other tests *in vitro*, the anaphylactic luetin test cannot be regarded as an index of resistance to *treponema pallidum*; these experiments and the failure of others (Neisser and Bruck; Nakano and Noguchi) to produce active immunity in animals to syphilis indicate that a lytic type of immunity is absent in syphilis or, at least, that it plays but a minor rôle in this infection.

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THE RELATION OF THE DIPHTHERIN SKIN REACTION TO IMMUNITY IN DIPHTHERIA¹

ANAPHYLACTIC SKIN REACTIONS IN RELATION TO IMMUNITY. III

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Since the clinical diagnosis of diphtheria is made so readily with the aid of cultures and as resistance to this infection is so largely antitoxic in nature, anaphylactic skin tests with diphtheria protein appear not to have been made until recently, because they are uncalled for in diagnosis and studies in this disease, therefore, have been mainly devoted to the toxin and its antitoxin. As diphtheria was among the first of the infections to be treated with horse immune serum, anaphylactic reactions due to the protein of the serum were among the earliest observations on anaphylactic phenomena, but these reactions bear no relation to the possibility of hypersensitiveness to the protein of the diphtheria bacillus itself.

Recently the possibility of eliciting a cutaneous anaphylactic reaction with the diphtheria protein has been pointed out by Park, Zingher and Serota (1), who, in conducting the Schick toxin test for immunity in diphtheria, noted that a small percentage of persons presented a reaction readily differentiated from the true toxin reaction, which they regarded as an anaphylactic response to the protein of the broth used in the preparation of the toxin and to the protein of autolyzed diphtheria bacilli. These reactions were described by Park and his associates as being of earlier development, more infiltrative, less sharply circum-

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scribed, faintly or not at all pigmented and usually not followed by scaling of the skin.

In a study of the reactions in the skin following the intracutaneous injection of varying amounts of protein and other substances in broth alone, Kolmer and Moshage (2) found that trauma is an important factor, particularly among those persons whose skins are unusually sensitive as is likely to be found among persons suffering with scarlet fever and measles. The reactions were usually slight and poorly colored and the majority subsided within forty-eight hours after injection.

Recently I have studied the true anaphylactic skin reaction with an emulsion of a large number of neutralized, washed and heat killed cultures of diphtheria bacilli (*diphtherin*), the method of preparing and conducting the tests and the results observed being given in other communications (3, 4). Typical anaphylactic reactions were observed that were entirely independent of the true toxin reaction of Schick, and it is highly probable that the majority of anaphylactic reactions following the intracutaneous injection of diphtheria toxin in conducting the Schick test are due to hypersensitiveness to the protein of the diphtheria bacillus.

While immunity in diphtheria appears to be largely antitoxic in nature, the probable relationship of the cutaneous anaphylactic reaction to immunity in this infection possesses considerable interest especially in view of the hypothesis that cutaneous anaphylactic reactions may be regarded as an index to immunity.

It is highly probable that antibodies other than antitoxin may be produced following immunization or infection with diphtheria toxin, the bacilli or both. Bandi (5) claims to have produced an antibacterial serum; Lipstein (6) failed to demonstrate bacteriolysins in immune sera as ordinarily prepared with toxin and believes the action of such sera to be purely antitoxic. Martin (7) found immune sera to contain both bacteriolysins and agglutinins; Martin, Prévot and Loiseau (8) and Sadikova (9) also have reported agglutinins in the sera of horses immunized with toxin. Complement fixing antibodies in the sera of im-

munized animals or of persons that have had diphtheria or that are carriers of diphtheria bacilli have been reported by Lambotte (10), Ponjol and Delanoe (11), Armand-Delille (12), Weill-Hallé and Bloch-Michel (13) and Cathoire (14), Kolmer (15) found that complement fixation occurred with the sera of immunized horses and antigens of diphtheria bacilli, but seldom with the sera of normal persons or those convalescent from diphtheria. Morse (17) found complement-fixing antibodies in the sera of animals immunized with the bacilli; Priestly's (18) attempts to show the presence of these antibodies failed owing probably to poor antigens.

The object of this investigation was to study the significance of the cutaneous anaphylactic reaction with diphtheria protein to lytic-immunity in diphtheria, and particularly in relation to the presence of bacteriolysins, agglutinins and complement-fixing antibodies for the diphtheria bacillus in the sera of persons yielding positive and negative skin tests.

METHOD OF STUDY

Sera. Out of a large number of persons in whom the *diphtherin* and toxin skin tests had been conducted, 57 of those that reacted positively and negatively to the *diphtherin* test in a typical manner were selected and their sera used for bacteriolytic, agglutination and complement-fixing tests. These persons reacted positively or negatively to the toxin test as recorded in the various tables. In a number of instances the sera of persons reacting negatively in the toxin test were also titrated for their content of natural diphtheria antitoxin. In addition to these the sera of five normal and five diphtheria antitoxin horses were studied in the same manner.

The *diphtherin* test was made by injecting intracutaneously 0.1 cc. of the prepared emulsion of diphtheria bacilli; the toxin tests were conducted with a similar injection of $\frac{1}{40}$ M.L.D. of a strong toxin diluted with sufficient sterile salt solution so that this dose was contained in 0.1 cc. All reactions were read 48 hours after injection.

Blood was collected aseptically from a vein at the elbow and the serum was used in the bacteriolytic tests within sixteen hours after collection.

Bacteriolytic tests. For testing the bacteriolytic power of the sera

for the diphtheria bacillus, two methods were employed, namely, a modification of the looped pipet method of Wright (19) and the method of Stern and Korte.

In the former method each serum was used fresh in order to utilize the native complements and the bacteriolytic power was measured by using a constant volume of undiluted serum with varying numbers of bacilli. The particular advantage of this method consists in applying tests *in vitro* under conditions more nearly approaching conditions *in vivo*; namely, that the person's own complements are used and the content of bacteriolysin is roughly measured according to the approximate number of bacilli killed.

Hiss serum water media containing 1 per cent dextrose and colored with litmus was used as the culture medium in the pipets in conducting the tests and various controls.

Cultures of the diphtheria bacillus were used undiluted, and after dilution with sterile broth 1:10, 1:100, 1:500, and 1:1000; the approximate number of bacilli in each was determined by plating 0.1 cc. of each in ascites dextrose agar. The number of living bacilli in these emulsions varied in different experiments, so that in the tables composed of tests made at different times, the upper and lower limits are given.

In the method of Stern and Korte, all sera were inactivated and complement furnished by fresh sterile guinea pig's serum. Five dilutions of serum were used: namely, 1:10, 1:20, 1:40, 1:80 and 1:160. Ascites dextrose agar was used as the plate medium in the tests and controls.

In both methods two cultures of the granular types of the diphtheria bacillus accustomed to grow in a fluid medium and capable of producing acid in twenty-four hours with dextrose were mixed and used. Cultures were grown in ascites broth for twenty-four hours and shaken mechanically with glass beads to break up clumps before use.

Agglutination tests. These were conducted with the emulsion of forty-five different cultures of washed and heat killed diphtheria bacilli (*diphtherin*). The macroscopic technic was employed with various dilutions of sera and the readings made after two hours incubation and further standing at room temperature for six hours.

Complement-fixation tests. In these tests *diphtherin* was used as the antigen in amounts corresponding to one-third the anticomplementary unit, as determined by preliminary titration.

With all sera the Wassermann reaction was likewise included and three antigens employed; namely, an alcoholic extract of heart re-

enforced with cholesterin; an alcoholic extract of syphilitic liver and an extract of acetone-insoluble lipoids of human heart.

All sera were heated at 56°C. for half an hour and used in a constant dose of 0.2 cc.

The antisheep hemolytic system was employed with the pooled sera of two or more guinea pigs for complement; in the antigen titrations and main tests two units of hemolysin were used and the results were read immediately after the second period of incubation.

Antitoxin titrations. For titrating the amount of natural diphtheria antitoxin in human serum a toxin was so diluted that 1 cc. contained one-fortieth of the L + dose. By mixing 0.2 cc. of patient's serum with 0.2 cc. of toxin, allowing the mixture to stand at room temperature for thirty minutes and injecting 0.1 cc. of it intracutaneously in the abdominal surface of a guinea-pig (prepared by pulling out the hairs) a test was made for one-fortieth unit of antitoxin to each cubic centimeter of serum. By using twice the quantity of serum a test is made for one-eightieth unit and by so varying the quantities of serum and toxin in this manner quantities of antitoxin varying from 10 units to $\frac{1}{320}$ unit may be determined.

From two to four injections were made into each pig and the results were read after two to four days.

RESULTS

I. Bacteriolysis in vitro in relation to the anaphylactic skin reaction to diphtherin

The results of bacteriolytic tests observed with the sera of persons reacting positively and negatively to the *diphtherin* test are shown in Table 1.

In no instance were there any evidences of a bactericidal action of sera of persons reacting positively or negatively to the anaphylactic skin test upon the cultures of diphtheria bacilli even when the number of living bacilli had been as few as 300 per cubic centimeter of emulsion.

It will be noted that the sera of persons reacting negatively to the toxin test contained $\frac{1}{40}$ or more units of diphtheria antitoxin; these results are similar to those observed by Schick, Park, Kolmer and Moshage, and others.

The results of bacteriolytic tests after the method of Stern and Korte (employing guinea-pig serum complement) with the sera of a second group of persons, are shown in table 2.

With the lowest dilution of serum there was apparent in some instances a feeble bactericidal effect as based upon a reduction

TABLE 1

Bacteriolytic tests after the method of Wright and antitoxin content of the sera of persons yielding positive and negative diphtherin and toxin reactions

NO.	AGE	SKIN REACTIONS		ANTITOXIN IN UNITS PER CUBIC CENTIMETER OF SERUM	BACTERIOLYTIC TESTS					
		Diphtherin	Schick		Uncount* per cc.	75,000 to 90,000 per cc.	15,000 to 25,000 per cc.	8000 to 2000 per cc.	1000 to 300 per cc.	Serum control
	years	cm.	cm.							
1	6	0.6 × 0.6	—	about $\frac{1}{20}$ unit	+	+	+	+	+	—
2	6 $\frac{1}{2}$	—	1.0 × 1.2	None	+	+	+	+	+	—
3	6	1.0 × 1.0	0.6 × 0.6	about $\frac{1}{20}$ unit	+	+	+	+	+	—
4	9	1.0 × 1.2	—	$\frac{1}{10}$ unit	+	+	+	+	+	—
5	8	—	—	$\frac{1}{20}$ unit	+	+	+	+	+	—
6	5	1.0 × 0.6	—	$\frac{1}{10}$ unit	+	+	+	+	+	—
7	22	2.0 × 3.0	—	$\frac{1}{20}$ unit	+	+	+	+	+	—
8	26	—	—	$\frac{1}{20}$ unit	+	+	+	+	+	—
9	24	1.0 × 1.5	1.0 × 0.8	None	+	+	+	+	+	—
10	19	1.0 × 1.8	—	$\frac{1}{20}$ unit	+	+	+	+	+	—
11	26	2.0 × 1.5	—	$\frac{1}{40}$ unit	+	+	+	+	+	—
12	30	—	0.5 × 0.8	None	+	+	+	+	+	—
13	28	Doubtful	—	$\frac{1}{10}$ unit	+	+	+	+	+	—
14	31	—	—	1 unit	+	+	+	+	+	—
15	24	—	—	$\frac{1}{5}$ unit	+	+	+	+	+	—
16		Culture	0	0	+	+	+	+	+	0

* Number of diphtheria bacilli per cubic centimeter of twenty-four hour ascites broth cultures employed.

— = negative skin test or sterility in the bacteriolytic tests.

+

in the number of colonies as compared with the control; these results, however, bore no relation to the results of skin tests.

Table 3 shows the results of bactericidal tests with the sera of a third group of persons reacting positively and negatively to the anaphylactic skin test, conducted in this manner:

In a series of sterile test tubes were placed 0.2 cc. of fresh sterile serum and increasing amounts of an ascites broth culture of the diphtheria bacillus; the total volume in all tubes including the serum control was made 1 cc. by the addition of sterile salt solution. After mixing and incubating at 37°C. for two hours, 5 cc. of ascites dextrose broth was added to all tubes, mixed and re-incubated for forty-eight hours when the results were noted after microscopic examination of the growths in each tube to exclude the possibility of contamination.

TABLE 2

Bacteriolytic tests after the method of Stern and Korte and antitoxin content of the sera of persons yielding positive and negative diphtherin and toxin reactions

NO.	AGE	SKIN REACTIONS		ANTITOXIN IN UNITS PER CUBIC CENTIMETER OF SERUM	SERUM DILUTIONS					
		Diphtherin	Schick		1:10*	1:20	1:40	1:80	1:160	Serum control
	years	cm.	cm.							
1	6	1.0 × 1.0	—	$\frac{1}{10}$ unit	slight	no	no	no	no	—
2	9	1.0 × 0.6	—	$\frac{1}{10}$ unit	slight	no	no	no	no	—
3	8	—	—	$\frac{1}{8}$ unit	no	no	no	no	no	—
4	5	0.6 × 0.6	0.6 × 0.6	about $\frac{1}{20}$ unit	no	no	no	no	no	—
5	6	1.0 × 1.0	—	$\frac{1}{10}$ unit	slight	no	no	no	no	—
6	9	1.0 × 0.6	0.6 × 0.4	None	slight	slight	no	no	no	—
7	4	—	1.0 × 1.0	None	slight	no	no	no	no	—
8	3	1.0 × 1.0	—	$\frac{1}{10}$ unit	no	no	no	no	no	—
9	5	0.6 × 0.6	—	$\frac{1}{10}$ unit	slight	no	no	no	no	—
10	9½	1.0 × 1.2	0.4 × 0.6	None	slight	no	no	no	no	0

* Slight = slight reduction in the number of colonies as compared with controls.

No = no reduction in the number of colonies.

— = negative skin test or sterility.

As will be noted in the table none of these sera, regardless of the results of the skin test, showed any evidences of bactericidal power in these experiments.

It was considered of interest to apply the *diphtherin* tests to normal and toxin injected horses and conduct similar tests with their sera.²

² I am indebted to Dr. C. P. Brown, for conducting the anaphylactic tests and furnishing sterile blood from a number of normal and immune horses; also to Dr. C. Y. White for the blood of several normal and immune horses.

The *diphtherin* tests were conducted by intrapalpebral injections of 0.08 to 0.1 cc. of the same *diphtherin* as used throughout the study.

The blood of each animal was collected aseptically and the bacteriolytic tests finished within five hours after bleeding.

The results of these tests are shown in tables 4 and 5.

TABLE 3

Results of bactericidal tests on B. diphtheria with the sera of persons reacting positively and negatively to the diphtherin skin test

NO.	SERA FROM ADULTS (DIPHTHERIN REACTIONS)	BACTERICIDAL TESTS (0.2 CC. SERUM)				
		0.1 cc.* culture	0.2 cc. culture	0.3 cc. culture	0.4 cc. culture	Serum control
	<i>cm.</i>					
1	2.0 × 3.0	+	+	+	+	—
2	—	+	+	+	+	—
3	1.0 × 1.5	+	+	+	+	—
4	1.0 × 1.8	+	+	+	+	—
5	2.0 × 1.5	+	+	+	+	—
6	—	+	+	+	+	—
7	Doubtful	+	+	+	+	—
8	—	+	+	+	+	—
9	—	+	+	+	+	—
10	3.0 × 4.5	+	+	+	+	—
11	2.0 × 1.8	+	+	+	+	—
12	1.5 × 1.2	+	+	+	+	—
13	—	+	+	+	+	—
14	2.0 × 1.5	+	+	+	+	—
15	Controls	+	+	+	+	0

* Twenty-four hour ascites broth culture of *B. diphtheria*; 0.1 cc. plated in ascites agar showed innumerable colonies.

The dash (—) indicates a negative skin test and sterile results in the bactericidal tests; the plus sign (+) indicates good growths and absence of bacteriolytic action of the sera.

The results of bacteriolytic tests shown in table 4 were conducted after the method of Wright, as previously described, the complements in the serum of each horse being thereby utilized; the tests shown in table 5 were also conducted with fresh active sera after the method described above in explanation of the results shown in table 3.

TABLE 4

Results of anaphylactic and bactericidal tests (method of Wright) among normal and diphtheria-antitoxin horses

NO.	HORSE	DIPHTHERIN TEST	BACTERIOLYTIC TESTS*					
			Un- count per cc.	70,000 to 100,000 per cc.	20,000 to 30,000 per cc.	10,000 to 3,000 per cc.	1500 to 400 per cc.	Serum control
2843	Normal.....	Negative	+	+	+	+	+	-
2833	Normal.....	Negative	+	+	+	+	+	-
2836	Normal.....	Negative	+	+	+	+	+	-
2500	Immune†.....	Negative	+	+	+	-	-	-
2614	Immune.....	Negative	+	+	+	+	-	-
2616	Immune.....	Negative	+	+	+	-	-	-
0	Culture control...	0	+	+	+	+	+	0

* Number of diphtheria bacilli per cubic centimeter of twenty-four hour ascites broth cultures employed.

† These horses contained about 350 to 850 units of antitoxin per cubic centimeter of serum at the time these tests were made.

+ = growth.

- = sterile.

As shown in table 4, the sera of the antitoxin horses showed a feeble bactericidal activity as determined by the pipet method; in the second method (table 5) where the number of bacilli was larger there was no appreciable bactericidal activity. In all instances the *diphtherin* tests were regarded as negative.

II. Protection in relation to the anaphylactic skin reaction to diphtherin

In view of the limited value and dependability of tests *in vitro* for antibodies and particularly those of a lytic nature, such as the bacteriolysins, in determining the presence or absence of a state of actual immunity in relation to the condition of hypersensitiveness, actual protection experiments are to be regarded as more decisive and reliable. During the past year while this study was in progress three persons that yielded well marked skin reactions to *diphtherin* contracted diphtheria; all of these

reacted positively to the Schick test and showed thereby an absence of natural antitoxin in the body fluids.

While these experiences may not demonstrate in a positive manner that the state of hypersensitiveness to diphtheria protein, as shown by the positive *diphtherin* tests, cannot be taken as an index of immunity, because diphtheria is essentially a toxemia and the skin reactions are probably due to a mechanism entirely distinct from that of the toxin-antitoxin reaction, they have shown quite conclusively that from a practical standpoint the anaphylactic skin reaction possesses no value in determining the presence or absence of actual immunity to diphtheria.

TABLE 5

*Results of bactericidal, agglutination and complement-fixation tests with the sera of normal and diphtheria immune horses on B. diphtheria**

NO.	HORSE†	AGGLUT.‡ 1:2 TO 1:160 (DIPHThERIN)	COMPLEMENT-§ FIXATION (DIPHThERIN)	BACTERICIDAL TESTS (SERUM 0.2 CC.)				
				0.1 cc. culture	0.2 cc. culture	0.3 cc. culture	0.4 cc. culture	Serum control
1	Normal (2843)....	—	—	+	+	+	+	—
2	Normal (2833)....	1: 2	—	+	+	+	+	—
3	Normal (2836)....	1: 2	—	+	+	+	+	—
4	Normal.....	—	—	+	+	+	+	—
5	Normal.....	—	—	+	+	+	+	—
6	Immune (2500)...	1: 16	+	+	+	+	+	—
7	Immune (2614)...	1: 32	—	+	+	+	+	—
8	Immune (2616)...	1: 8	++	+	+	+	+	—
9	Immune.....	1: 16	—	+	+	+	+	—
10	Immune.....	1: 16	—	+	+	+	+	—
11	Culture control..	0	0	+	+	+	+	0

* Twenty-four hour ascites broth culture; 0.1 cc. plated in ascites agar showed an uncountable number of colonies after twenty-four hours incubation.

The dash (—) indicates a negative reaction or sterile serum; the plus sign (+) indicates good growths and absence of bactericidal activity.

† The *diphtherin* tests (intrapalpebral injection) were negative among all horses in which this test was made.

‡ The dilution expressed is the highest in which well marked agglutination was observed.

§ Wassermann reactions were negative with all antigens.

III. Agglutination and complement-fixation in relation to the anaphylactic skin reaction to diphtherin

The results of agglutination and complement-fixation tests with the sera of persons reacting positively and negatively to the skin tests and normal and immunized horses (reacting negatively to the anaphylactic skin test), are shown in tables 5 and 6.

TABLE 6

Complement-fixation and agglutination reactions with the sera of persons yielding positive and negative diphtherin and toxin skin reactions

NO.	AGE	SKIN REACTIONS		COMPLEMENT-FIXATION TESTS		AGGLUTINATION TESTS					
		Diphtherin	Schick	Diphtherin	Wassermann	1:10	1:20	1:40	1:80	1:160	1:320
	<i>years</i>	<i>cm.</i>	<i>cm.</i>								
1	9½	—	0.6 × 1.0	—	—	—	—	—	—	—	—
2	30	—	—	—	—	—	—	—	—	—	—
3	6*	1.0 × 1.0	—	—	—	±	—	—	—	—	—
4	9*	1.0 × 1.0	—	+	—	—	—	—	—	—	—
5	8*	1.0 × 0.6	—	±	—	+	±	—	—	—	—
6	6*	1.0 × 1.2	—	—	—	—	—	—	—	—	—
7	5*	0.6 × 0.6	0.4 × 0.6	—	—	—	—	—	—	—	—
8	3	0.3 × 0.5	—	—	—	±	—	—	—	—	—
9	17	—	—	—	—	—	—	—	—	—	—
10	24	—	—	—	—	—	—	—	—	—	—
11	42	1.0 × 1.0	—	—	—	±	—	—	—	—	—
12	46	—	—	—	—	—	—	—	—	—	—
13	9	0.9 × 1.2	—	—	—	—	—	—	—	—	—
14	28	1.0 × 1.0	—	±	—	—	—	—	—	—	—
15	43	1.0 × 0.6	—	—	—	—	—	—	—	—	—
16	22	1.0 × 0.4	—	—	—	—	—	—	—	—	—

* Received 1250 units of antitoxin 6 weeks before these tests were made.

The dash (—) indicates a negative and the plus sign (+) a positive reaction.

A small percentage of the sera of normal persons was found to give partial agglutination of the *diphtherin* in the lowest dilution of serum (1:10) and all of these reactions occurred with the sera of persons yielding positive skin tests.

A few of the sera of normal horses were found to agglutinate the *diphtherin* in dilutions of 1:2, but not in higher dilutions; all of the sera of the diphtheria-antitoxin horses caused agglu-

tion in dilutions ranging from 1:8 to 1:32, but not in higher dilutions. As previously stated, all of the horses tested for the *diphtherin* anaphylactic reaction yielded results interpreted as negative.

The majority of human sera reacted negatively in complement-fixation tests employing *diphtherin* as antigen; the few weakly positive reactions observed occurred among persons reacting positively in the skin test (table 5). The sera of all normal horses yielded negative reactions; two of five antitoxin horses, however, yielded positive reactions, which confirm previous work and indicates that during toxin immunization both agglutinins and complement-fixing antibodies are produced along with antitoxin.

CONCLUSIONS

1. Bacteriolysins for *B. diphtheria* could not be demonstrated in the sera of normal persons.
2. The sera of persons yielding positive reactions to the cutaneous *diphtherin* anaphylactic test were without demonstrable bactericidal effect upon *B. diphtheria*.
3. Persons reacting positively in the *diphtherin* anaphylactic test may contract clinical diphtheria.
4. Normal horse serum possesses no demonstrable bactericidal action over *B. diphtheria*.
5. The sera of horses immunized with diphtheria toxin may contain small amounts of a bactericidal antibody in addition to antitoxin; apparently these bactericidal substances may be present in the blood serum of horses without well defined hypersensitiveness to the diphtheria protein.
6. A condition of hypersensitiveness to diphtheria protein may exist without demonstrable amounts of agglutinin or complement-fixing bodies for *B. diphtheria* in the body fluids.
7. The results of this study indicate that hypersensitiveness to diphtheria protein cannot be taken as an index to immunity to diphtheria and that the anaphylactic state bears no constant nor direct relation to the presence or absence of such antibodies as may be detected by experiments *in vitro*.

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THE ANTIGENIC PROPERTIES OF TUBERCLE WAX

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Tubercle wax is the name applied to fatty and waxy substances extracted from tubercle bacilli with fat solvents such as alcohol, ether, benzol, chloroform, etc.

HISTORICAL AND NATURE OF TUBERCLE WAX

Hammerschlag was first to show that tubercle bacilli contained a large amount of fatty and waxy substances, and that in this respect they differ from other vegetable microorganisms. De Schweinitz and Dorset confirmed Hammerschlag's results, and since then a great deal of attention has been paid to this subject. All investigators agree that it is difficult to extract the fatty substances entirely, and that the amount is influenced by the culture media upon which the bacilli are grown and by other factors mentioned below. Proskauer and Beck have demonstrated that bacilli cultivated on fat free media are also able to produce wax, although in smaller amount (10 per cent of dried bacillary substance). Klebs, R. Koch and others have shown that the peculiar staining reaction of tubercle bacilli depends upon this fatty matter, and that the extracted fat will become deeply colored with carbolfuchsin, and retain this color when subsequently treated with dilute mineral acids. In other words tubercle bacilli are "acid fast" because of the fatty and waxy matter they contain. The same authors have also shown that after extraction of this matter the bacilli are no longer "acid fast." Aronson demonstrated that the chief amount of the wax is not contained in the bodies of the microorganism, but lies between them, and that it is probably a product of secretion of the bacilli.

The nature of tubercle wax has not been as yet definitely determined. Hammerschlag found tripalmitin, tristearin and lecithin but no cholesterin. According to Kresling the fatty matter contains 14.38 per cent of free fatty acids, 77.25 per cent of neutral fats and fatty acid esters, and 0.37 per cent of water soluble material. He also demonstrated the presence of lecithin (0.16 per cent) and cholesterin. R. Koch extracted two substances. One was soluble in cold alcohol and the other soluble only in hot alcohol or ether. Both were acid fast, but he believes that the second substance is chiefly responsible for this property of the tubercle bacilli. This substance was shown by Aronson to be a true wax, which was confirmed by Ruppel. Ruppel extracted three kinds of fat: (1) with cold alcohol, a greasy red coloring matter, which exists in the bacillary bodies as chromogen and obtains its color through contact with the air; (2) with hot alcohol, a waxy substance; (3) with ether, a wax.

PERCENTAGE PRESENT

The percentage of fatty and waxy material that can be extracted varies according to the different strains of the tubercle bacilli, their age and virulence, the media upon which they are grown and the solvents employed.

De Schweinitz and Dorset extracted various cultures, grown upon the same media with the following amounts of fatty material: virulent human bacilli 28.03 per cent; attenuated human bacilli 37.41 per cent; bovine bacilli 26.32 per cent; swine bacilli 20.59 per cent; horse bacilli 31.76 per cent; avian bacilli 30.65 per cent. Hammerschlag obtained 26.2 per cent on the average (alcohol-ether); Aronson 20 to 25 per cent, Levene 31.56 per cent, Ruppel 8 to 10 per cent as a minimum, 25 to 26 per cent as a maximum; Klebs, 20.5 per cent with ether and 1.14 per cent more with benzol; Kresling, 36 per cent (chloroform), 34.31 per cent (benzol), 30.75 per cent (ether), 24.76 per cent (alcohol) with the use of alcohol, ether and chloroform in succession he obtained on the average 38.95 per cent fatty substances.

OBJECT OF PRESENT INVESTIGATION

The object of this investigation was two fold:

1. To determine whether extract of tubercle wax possess any antigenic value in the serum diagnosis of tuberculosis.
2. To explain the reason for the frequent fixation of luetic sera with various antigens used in complement fixation in tuberculosis.

PREVIOUS WORK

Several investigators have used alcohol, or alcohol-ether extracts of tubercle bacilli as antigen.

K. Meyer found that the fat, fatty acids and wax like fractions of tubercle bacilli possess slight or no fixing properties for tuberculosis antibodies, using Hoechst anti-tuberculosis serum. Caulfield and Beatty used an alcohol-ether extract of a young culture and obtained deviation of complement in a certain percentage of cases of tuberculosis, and no fixation in non-tuberculous cases. Dudgeon, Dudgeon, Meek and Weir and Meek used an alcoholic extract of tubercle bacilli and obtained a high percentage of fixation in tuberculosis. All of the above mentioned investigators used young cultures. It is to be noted here that tubercle wax is not present in any considerable amount in young cultures but develops only after the organisms have been grown for some time. It is doubtful, therefore, whether the substance was present in appreciable amount in their antigens. (Paul Ehrlich was the first to show that in young cultures of the tubercle bacilli many of the organism are not acid fast. Aronson found that in young rapidly growing cultures the wax is not present.)

As to the fixation of the sera possessing lipotropic properties (luetic sera being the most important) this heretofore has been attributed to the high lipid content of the egg media upon which the bacilli were usually cultivated in the preparation of the tuberculins (antigens). J. Bronfenbrenner extracted the lipin contained in one such antigen, that of Besredka, and found that emulsion of these lipins caused deviation of complement with luetic sera. Here again it was not certain whether this was due to lipoids derived from the media or from the bacilli. There are

also antigens prepared from cultures of tubercle bacilli not grown upon egg containing media, which also cause fixations with syphilitic sera. It suggested itself that the waxy substances of the tubercle bacilli might cause such fixations and various extracts were prepared from tubercle wax and used with sera of normal,luetie and tuberculous patients.

MATERIALS AND METHODS OF STUDY

Tubercle wax. The tubercle wax was kindly furnished by Dr. Rucker, of the Pennsylvania State Laboratories. It was prepared by growing both human and bovine strains in glycerin bouillon for several months, separating the bacillary bodies by filtration and extracting them in a Soxhlet apparatus, first with absolute alcohol for two to three hours, then with ether for one hundred and sixty to one hundred and eighty hours. The wax is precipitated from the ether distillate with absolute alcohol and redissolved in hot benzol. This process is repeated twice. The wax as thus obtained, is of a light yellowish brown color and of soft consistency and it leaves a slight fat stain on paper. It resembles somewhat dried cerumen. Tubercle bacilli extracted by the above method yield about 20 to 22 per cent wax.

Antigens. After preliminary experiments it was found that emulsions prepared from ether, or ether-methyl alcohol solutions by the were most suitable for this investigation.

Antigen A. Fifty milligrams of tubercle wax were dissolved in 0.5 cc. of ether by agitation in a stoppered test tube. To this was gradually added 25 cc. of normal salt solution containing 0.5 per cent phenol. The mixture was repeatedly shaken for several hours. Coarse waxy particles separated, which were removed by filtration through cotton. The resulting antigen was slightly cloudy.

Antigen B. To the ether solution prepared as above 1 cc. of methyl alcohol was added. This caused a milky white emulsion, which was diluted with 25 cc. of normal salt solution, containing 0.5 per cent phenol, repeatedly shaken during several hours and filtered through cotton. The resulting antigen was slightly milky.

The antigens were prepared on the same day that the tests were made, since it was found that they soon became anti-complementary. In the anti-complementary titration increasing doses of antigen (0.01; 0.05; 0.1; 0.2; 0.4; 0.6; 0.8; 1.0; 1.5; 2.0 cc.) were added to a constant amount of guinea pig complement. The volume in the tubes was made approximately equal by the addition of normal saline and the mixtures were incubated for one hour at 37°C. Then corpuscles and two units of hemolysin were added. The results were read after a second incubation of one hour. The anti-complementary dose was taken as the smallest amount of antigen, causing any inhibition of hemolysis. One-third to one-half of this amount was used in the tests. The anti-complementary units of the antigens varied considerably with the serum of different guinea-pigs. As a rule antigen A was slightly anti-complementary in doses of 1.0 cc., 0.3 cc. being usually the amount employed in the tests. Antigen B was usually anti-complementary in doses of 0.8 cc, 0.25 cc. being then used. When the anti-complementary dose was found to be higher or lower the amounts used were proportionate. Antigen B, while more anti-complementary, yielded somewhat sharper results. The antigens were also titrated with luetic sera, but it was found that fixation varied so greatly that this procedure was finally abandoned.

Hemolytic system. Anti-sheep hemolysin of a high titer was used. This was titrated each time before the tests were made with 0.5 cc. of a 10 per cent dilution of fresh guinea-pig serum and the same amount of the 5 per cent suspension of washed sheep corpuscles. Usually the pooled serum of two or more animals was used. Two units of hemolysin (one hour's incubation) were employed in the tests.

Sera. All sera were inactivated for thirty minutes at 56°C. and used in a constant dose of 0.1 cc.

Technic. Inactivated serum, antigen and complement in the amounts stated above were incubated for one hour, then corpuscles and hemolysin were added and the results read after another hour's incubation. The controls were the usual ones employed in complement fixation tests. A serum control was

used for each serum. All sera were tested with both tubercle wax antigens, and one or more cholesterinized alcoholic extracts of human heart. The latter extracts were antigenic in doses of 0.05 cc. of a 1 to 20 dilution; 0.2 cc. was used as the antigenic dose.

SUMMARY OF RESULTS

Two hundred sera were examined in all.

Twenty sera were from clinically certain cases of pulmonary tuberculosis (various stages). Four reacted strongly positive to the Wassermann test. Of these, three also deviated complement with the tubercle wax antigens. Three sera not reacting to the Wassermann reaction, reacted positively with the tubercle wax antigen, the other sera did not cause fixation of complement. Seventy-six luetic, and presumably non-tuberculous sera reacted positively with the Wassermann reaction. Of these fifty-nine also deviated complement with the tubercle wax antigens, while seventeen showed complete hemolysis. (Some of the negative cases showed only slight inhibition of hemolysis with the syphilitic antigen.)

One hundred and four sera were from presumably non tuberculosis and non syphilitic cases. Of these, seventeen reacted positively with tubercle wax antigens and eighty-seven negatively. The inhibition of the hemolysis with tubercle wax antigen was only in a few cases as marked as with the syphilitic antigens. It varied usually from slight to partial inhibition and as a rule was more marked with antigen B.

TABLE I
Showing results obtained with tuberculous, luetic and normal sera

SERA	NUMBER EXAMINED	ANTIGEN A AND B TUBERCLE WAX		+ WASS. REACT.	- WASS. REACT.
		+ Reactions	- Reactions		
Tuberculous.....	20	6	14	4	16
Luetic.....	76	59	17	76	0
Normal.....	104	17	87	0	104
Total.....	200	82	118	80	120

TABLE II

Showing comparative results with tubercle wax antigens and cholesterinized antigen (W.R.)

82 TBWA +		118 TBWA -		80 WR +		120 WR -	
WR+	WR-	WR+	WR-	TBWA+	TBWA-	TBWA+	TBWA-
62	20	18	100	62	18	20	100

T.B.W.A. = Tubercle wax antigen (A and B)

W.R. = Wassermann reaction.

CONCLUSIONS

1. Tubercle wax antigens cause complement deviation with a high percentage of sera possessing lipotropic properties.

2. These antigens also cause complement deviation with a moderate percentage of presumably normal and tuberculous sera. These sera may be derived from latent cases of syphilis although they fail to react positively with the Wassermann test.

3. They appear to possess neither diagnostic nor prognostic value in the serum diagnosis of tuberculosis.

I desire to thank Prof. Allen J. Smith for suggesting the subject, Dr. Rucker for furnishing the tubercle wax, Miss Willa McNitt for furnishing some of the sera and Dr. John A. Kolmer for many kind suggestions.

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PROCEEDINGS OF THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS

THIRD ANNUAL MEETING, HELD IN WASHINGTON, D. C.

May 11, 1916

The President, Dr. J. W. Jobling, in the chair.

The following officers were elected for the ensuing year: *President*, Richard Weil, New York City; *Vice-President*, John A. Kolmer, Philadelphia, Pa.; *Treasurer*, Willard J. Stone, Toledo, Ohio; *Secretary*, Martin J. Synnott, Montclair, N. J.

Members of the Council: Arthur F. Coca, New York City, term expires 1921; William H. Park, New York City, term expires 1920.

1. THE RELATION OF LIPOIDS TO IMMUNE REACTIONS¹

J. W. Jobling.

2. ADDITIONAL FACTS CONCERNING THE PROTEIN POISON

Victor C. Vaughan: Since the author's last publication on this subject the following facts have been ascertained by his students and himself:

1. Casein yields a large per cent of the protein poison.
2. The protein poison after the removal of all traces of mineral acid is strongly acid in and of itself.
3. The protein poison does not give the ninhydrin test, but does so after being split up with acid.
4. The poison gives a skin reaction in all persons.
5. The poison is not without harm when administered by mouth.
6. Animals may be acutely or chronically poisoned by oral administration.
7. In chronic poisoning by feeding, extensive fatty degeneration results.
8. The protein poisons from diverse proteins are not identical.
9. The protein poison from casein combines with certain unbroken proteins. In this combination the acidity of the poison is neutralized and its physiological action diminished.
10. From the tissues of animals killed with the protein poison, it may be extracted with acidified alcohol, and its presence demonstrated and amount roughly estimated by intravenous injections of guinea-pigs.

¹President's Address (see p. 491).

3. THE INADEQUACY OF THE ANAPHYLATOXIN THEORY OF ANAPHYLAXIS

Richard Weil: The discussion is limited to the consideration of anaphylatoxin in the sense originally intended by Friedberger; namely, the product of the incubation of antigen and antibody in the presence of serum. The characteristic features of the test tube reaction are (1) that it takes place not only through the interaction of an immunological couple, namely antigen and antibody, but of entirely unrelated sera; (2) That the two factors must be in certain definitely limited quantitative relationships; (3) that it is slow and gradual; (4) that it requires the presence of complement. In every one of these features it differs from the anaphylactic reaction in the living animal or in the suspended uterus.

The crucial test consists in the fact that it is impossible to produce the anaphylactic reaction in the animal by conditions which duplicate those in the test tube, namely the simultaneous intravenous injection of the two factors, antiserum and antigen.

According to the physical theory, the reaction is simply an expression of the alteration of cellular equilibrium which results when external antigen is brought into contact with cellular antibody. The characteristics of the reaction are all entirely in keeping with this interpretation. The precipitation reaction in the test tube, which is not accompanied by the chemical destruction of either factor, (1) is immediate; (2) proceeds in the absence of complement; (3) requires relatively large amounts of antibody and relatively minute amounts of antigen. In these respects it is perfectly analogous with the anaphylactic reaction. If, in place of the visible alteration, expressed as precipitation in the test tube, interaction of the two factors *in vivo* is supposed to produce an alteration of cellular equilibrium, such as will act as a cellular stimulus, all the requirements of the problem are satisfied. In view of the fact that precipitin has been demonstrated to be identical with the sensitizing antibody, this explanation of anaphylaxis seems almost self evident. This conception rids us of the necessity of postulating an intermediate chemical product, namely anaphylatoxin; such a postulate is not only superfluous, but it is also entirely incompatible with all of the characteristic features of the reaction.

The preceding discussion is limited to phenomena ascertained in guinea-pigs. Anaphylatoxin has been produced only through the incubation of guinea pig serum. Yet in the guinea pig the conclusion seems unavoidable that anaphylactic death is due to a cellular reaction, and that the serum changes play no role therein. There is no question, on the other hand, that during the prolonged or delayed shock of the dog, striking alterations occur in the chemical composition of the blood. These changes are due in the first instance to the freeing of protease, as a result of the reaction of the sensitized cells to the antigen. It still remains to determine whether the changes in the serum produced through the activity of the protease are really the cause of the later symptoms, or merely accompaniments of the shock in this species. Even in the

former event, the primary step in the entire reaction, namely the discharge of protease, would be cellular.

4. STUDIES REGARDING THE ACTION OF DIFFERENT BLOOD SERA UPON VARIOUS TISSUE SUBSTRATES

E. Abderhalden (presented by O. Berghausen).

5. THE SPECIFIC CHARACTER OF IMMUNITY REACTIONS

E. C. L. Miller: Ordinary immunity reactions may be considered as a result of training. A horse to produce diphtheria antitoxin must have been injected with diphtheria toxin, a child to be immune to scarlet fever must have had scarlet fever. This immunity is specific. The horse that has been injected with diphtheria toxin produces diphtheria antitoxin and not tetanus antitoxin; the child that has recovered from scarlet fever is immune to scarlet fever and not to measles. All our thinking about immunity is based on the assumption that the relation of antigen to antibody is specific. Medico-legal tests for blood have been worked out and accepted by the courts on the assumption that only one antigen could react with the same antibody. Now, however, there is evidence that this is not true, that two or more antigens may produce the same antibody and that the same antigen may produce two or more antibodies. This has been particularly well demonstrated in the hemolysis of the red blood cells of sheep. It has been shown by a number of workers that a specific sheep hemolysin can be produced in a rabbit not only by the injection of sheep blood cells but also by the injection of such entirely unrelated substances as the liver of the guinea pig, the gills of a carp or the kidneys of a turtle. The explanation of this seems to be that the relation of antigen to antibody does not depend on the entire protein molecule as a whole but rather on certain groups in the molecule and it so happens that various unrelated substances contain these groups and so produce a common antibody.

DISCUSSION OF THE PRECEDING FIVE PAPERS

H. Gideon Wells stated that in all problems of immunology, specificity is the vital point which can never be overlooked without disaster. Nevertheless, many hypotheses have been developed which entirely disregard specificity, and, therefore, must obviously be incorrect. In studying the principles of immunology there have been great difficulties because of the failure to grasp the essential principles, as laid down by Jacques Loeb—that in studying the fundamental principles of biological processes one must reduce the elements involved to the simplest possible, for at the best the reactions are complex and beyond our interpretation. It will be recalled that he reduced the factors in his reactions to the simplest possible, by using the single egg as a living organism and the simple inorganic salts as his agents. In studying the principles of immunology we should follow these principles as far as

possible. Unfortunately, we apparently cannot get below the whole protein molecule as one end of our reactions and generally must use the warm blooded mammals for the other side of the equation, although possibly work on cell cultures may help us to simplify our materials.

The best we can do, therefore, is to use pure protein, and, fortunately, there are some proteins that can be obtained in relatively pure condition. Those proteins, which differ greatly from the great majority of proteins, are especially favorable materials for purification, such as non-coagulable ovomucoid of egg white or alcohol soluble proteins of the grains. Using such isolated proteins, and others, we have found evidence that, delicate as the specificity of immunological reactions seems to be, immunological differences do not seem to occur between proteins that cannot also be differentiated chemically. The specificity differences of the different proteins seem to agree with differences in chemical composition, and, as yet, we have not found finer differences as might be expected, such as stereoisomeric differences with identical chemical composition.

To illustrate; by comparative study of different proteins isolated from hen's egg by anaphylaxis, I was able to distinguish five proteins definitely distinguishable from one another by this means. Osborne and Harris, working by chemical means, isolated from hen's egg five different proteins which corresponded exactly to the five antigens differentiated by anaphylaxis. Proteins of the egg that could not be differentiated by chemistry could not be differentiated by anaphylaxis.

Dr. Osborne and I have also found in many cases that proteins which are isolated by chemical means can be checked up very nicely as to their individuality by anaphylaxis and other immunological reactions, and immunological methods have been found to be of much help in establishing the chemical identity of unknown proteins.

John A. Kolmer said that it would be difficult to improve upon the excellent résumé presented by Dr. Jobling on the relation of lipoids to immunity. He agreed with the essayist that more and more importance is being attached to the role of lipoids in this field.

Dr. Kolmer stated that it has been amply proven that toxic substances may be prepared of various animal and vegetable proteins by the method employed by Dr. Vaughan; that likewise toxic substances could be produced in normal and immune sera by the addition of such substances as kaolin and agar capable of producing anaphylactic-like symptoms and lesions in experimental animals, but that it was not yet clear what relation these observations bore to the mechanism of anaphylaxis and particularly so in view of the work presented by Dr. Novy within the past few days. He asked Dr. Vaughan if he was prepared this morning to make any further statement in regard to the relation between his protein poison and the mechanism of anaphylaxis.

Dr. Kolmer asked Dr. Miller if he had made careful titrations of the content of antishoop hemolysin in the sera of his rabbits before immunization as the sera of a large proportion of these animals contain natural antishoop hemolysin.

Dr. Kolmer also stated that in his opinion "group reactions" in immunity were best explained at the present time according to the views expressed by Dr. Wells in his discussion.

Victor C. Vaughan said that he had watched Dr. Novy's experiments with the deepest interest. "Even before Doctor Novy spoke to me about it, I had observed the appearing and disappearing and reappearing wave of toxicity in serums being incubated with agar and other foreign bodies. I spent much time and sacrificed many animals in trying to measure these waves and catch the rhythm of the toxicity, but without results. I am not yet ready to abandon the idea that a protein poison is formed in anaphylactic shock. No one can tell whether this is due to a chemical or physical process. It is difficult to draw a line between physical and chemical changes. I can conceive that a body so complex as the protein molecule may be disassociated and a poisonous action developed even by high dilution. If so stable a body as sodium chloride can be broken up into its ions by dilution, is it not possible that even more marked alterations might occur in a highly complex molecule. I am convinced of the fact that the blood contains proteins from which a poisonous group is easily detached. Doctor Novy has accumulated much experimental data. I hope that he will soon publish his protocols and give all of us an opportunity to try our hand at theory and explanation."

James W. Jobling: As Dr. Weil states, recent work shows that the intoxicating dose in anaphylaxis probably acts first on the cells. In guinea-pigs it causes a contraction of the muscle cells of the bronchi to such a degree that immediate death ensues from asphyxia. In dogs, however, death does not occur for several hours, and the clinical picture is quite different. In the latter case we have observed definite changes in the blood, and we believe that death is probably due to the products of protein cleavage. The ferments, which are greatly increased in amount, are probably liberated as a result of the cell stimulation, and their activity is dependent upon colloidal changes that have taken place when the antigen is brought into contact with the serum. We observed a definite increase in the higher and lower protein cleavage products in the blood. These we believe are derived from the serum proteins and not from those introduced.

Richard Weil closed the discussion. He said that he was not particularly interested in establishing the universal validity of any of the current theories of anaphylaxis. Attempts of this sort had done more in the past to obscure the truth than to advance it. For example, the discovery by Friedberger that anaphylatoxin could be produced in the test tube led at once to the assumption that such a substance was produced *in vivo* and was the actual cause of death in the guinea pig, consequently the German school expended a great deal of labor to overthrow the cellular theory of anaphylaxis in the guinea pig and to establish the humoral theory in order to vindicate the truth of the primary assumption. Time, however, has completely established the truth of the cellular theory, so that in the guinea pig, at all events, it

seems certain that serum changes, with the production of so-called anaphylatoxin, can play no rôle in the typical evolution of shock. It would, however, be just as serious a mistake to assume that the process which takes place in the guinea pig must necessarily be universally applicable to the anaphylactic phenomenon throughout the animal kingdom. We know that as between the guinea pig and the dog, for example, certain striking differences exist. The white cells of the guinea-pig contain tryptic ferments, those of the dog contain none. Serum of the guinea pig is rich in proteolytic ferment; serum of the dog contains little, if any. Furthermore, it seems likely that the liver is essential to anaphylactic shock in the dog, whereas it plays no rôle in the guinea pig. Possibly, therefore, the mechanism in the two species is entirely different, as is further indicated by the rather striking differences in the anaphylactic symptoms themselves. We know with certainty that serum changes of chemical nature accompany anaphylactic shock in the dog. This fact, however, by no means argues that these changes are productive of the anaphylactic symptoms. Those symptoms may result from the gradual development of the cellular response to the antigen, taking place, however, more slowly and more gradually than in the guinea pig, just exactly as diphtheria toxin produces its effect slowly and gradually. In other words, as I have already stated, it still remains to determine whether serum changes in the dog, resulting in the production of some unidentified substance described as anaphylatoxin, are simply an accompaniment of anaphylactic shock in that animal, or are actually productive of the symptoms thereof.

6. THE PHENOMENON OF LEUCOCYTOSIS AND ITS IMPORTANCE AS A DIAGNOSTIC SIGN IN VACCINE TREATMENT¹

Joseph Head: I have taken 54 of my vaccine patients and made 714 separate blood counts containing complete data. Of these 54 cases 33 did not in the course of the treatment give a leucocyte count of over 10,000, but 21 cases did show transitory leucocyte counts of over 20,000 that jumped like a rocket from 5, 6, or 7,000 to 40, or 100,000, only in a few days to sink back to 8000 or 4000, with either no symptoms to speak of or an indisposition so slight as to be almost negligible. This series of cases is too small to warrant definite conclusions and should be corroborated by other observers before being accepted too seriously, yet they certainly indicate that a transient leucocyte count cannot be considered of itself an infallible sign of pus or even of semi-acute inflammation of a serious nature. Persistence of the leucocytosis accompanied by a great falling off of the red cells and hemoglobin would indicate a condition of an entirely different significance.

¹Subsequent to the presentation of this paper it was pointed out to Dr. Head that several of the highest leucocyte counts reported by him in different individuals were observed by the same person on the same day. This coincidence indicates that an error may have been introduced into all of those counts.—*Editor.*

Blood count work is of great value in determining vaccine dosage. In one or two cases in which the infections were chronic or general, the blood counts were of no value as a guide, but ordinarily they will give accurate sensitive warning of an oncoming reaction. The red cells may drop off as may the hemoglobin, the leucocytes may jump up or drop to two or three thousand, or a lymphocytosis of 50 per cent may develop. There is, however, no one special phase to be looked for as peculiarly diagnostic; it is the way the blood acts generally under the treatment that should be noted. If the hemoglobin, red cells and white cells are fairly normal in number, size and staining capacity or if the constituents of the blood under vaccine treatment show a steady improvement, the vaccine dosage can be maintained or increased, but if the dose of vaccine is followed either by leucocytosis or leukopenia or the red cells or hemoglobin drop off or if poikilocytes or nucleated red cells appear, the vaccine dosage should be watched with great caution, and if unfavorable symptoms develop the vaccine should be, at least temporarily, stopped.

Dr. Head announced his intention of endeavoring to corroborate his reports by more conclusive data, whereby team laboratory work would check his findings.

At the present time of writing Dr. Head is not convinced of the accuracy of his findings. He took his results to the meeting of the Society of Immunologists for information and advice.

7. THE ACTION AND THERAPEUTIC EFFECTS OF LEUCOCYTIC EXTRACT (ARCHIBALD). A PRELIMINARY REPORT

W. E. Richard Schottstaedt (presented by Willard J. Stone). The work has been carried on with leucocytic extract (Archibald) prepared from leucocytes obtained directly from the blood of normal animals. Subcutaneous injections in normal individuals and in patients suffering from acute infections produces a marked leucocytosis. The leucocyte increase is often 300 per cent, is highest within ten or twelve hours after the administration of the extract. The increase in the neutrophile elements is particularly marked and coincident with it there is a less marked increase in eosinophile cells.

Clinically, striking beneficial results in man have been obtained following its use in acute infections, such as furunculosis, pneumonia, acute bronchitis, acute tonsilitis. Chronic infections have had a less striking clinical improvement, though the leucocytic increase has been as marked as in the above cases.

DISCUSSION OF THE PRECEDING TWO PAPERS

Wm. Egbert Robertson: The results presented by Dr. Head are somewhat contradictory and very difficult of interpretation. As a general rule, any infection that gives rise to a leucocytosis causes at the same time a more or less pronounced anemia. Yet, according to his figures,

in many instances the blood count is relatively normal but for the often startling leucocyte count. I have had a large number of blood counts made in patients that presented more or less marked pyorrhea, but in no instance have I ever noted any approach to the extraordinary increase in the white cells to which Dr. Head calls attention. I am not sufficiently familiar with his work to justify any comment upon the possibility of technical errors, but I should be much inclined to doubt the leucocytosis recorded by him, particularly in some of the instances in those who have nothing more than a pyorrhea. Equally difficult of explanation, and I may add, equally improbable are the recorded differential counts, especially with respect to the percentage of eosinophiles. Though the genesis of eosinophilia is not definitely understood, we are fairly familiar with all of the conditions giving rise to it and among these we cannot include pyorrhea. We find it impossible therefore, to draw any definite conclusion from the results presented by Dr. Head, though the very elaborate and beautiful charts show a tremendous amount of earnest and very pains-taking work.

Concerning the leucocytic extract of Archibald and Moore, Dr. Robertson stated that he had never employed this. He further said that he had read an article by these authors, which was published in the Archives of Internal Medicine in 1914. The object of the authors was to produce a leucocytic extract with greater ease and in greater quantity than that obtained by Hiss and Zinsser. Manwaring also found that they were able to obtain very small amounts by injecting a 5 per cent suspension of aleuronat into the pleural cavities of rabbits. He, therefore, employed horses. Archibald and Moore, on the other hand, extracted blood under antiseptic precautions from the jugular vein of the horse or of some one of the domestic animals. This was immediately citrated and 0.5 of 1 per cent of acetic acid was added to destroy the red cells. The mixture was then centrifuged and the supernatant fluid was discarded. The sediment, which is rich in leucocytes, was washed several times in physiologic salt solution, ground with quartz and neutralized. Distilled water was then added to the sediment in the proportion of 4 to 1, heated at 58°C. for one hour and placed in an incubator until cytolysis was complete. It was again centrifuged, the supernatant fluid discarded and tri-cresol added for preservation. There is no doubt at all that these extracts have some value, but the trend today is away from specificity. For some years an occasional report has appeared in the literature, showing that non-specific split protein products yield results apparently quite equal to those that have been obtained by specific substances, notably bacterins. It must also be borne in mind that the best results follow intravenous injection. Dr. Robertson stated that Hiss and Zinsser's work suggested to him the use of purulent pleural exudates, particularly chronic cases with discharging sinuses, or cases which prove sluggish within a few weeks following operative interference. He inactivated the fluid at 56° for an hour and then injected 10 cc. This is not only a leucocytic extract, but in many instances also a bacterin. Only rarely did

abscess formation occur at the site of injection, but if Tessiae's fixation abscess is of any clinical value, then three possibilities are to be considered through which such a substance may effect beneficent results. (1) The fixation abscess, (2) The leucocytic extract, and (3) The bacterin.

This subject was referred to by Dr. Robertson in a paper read by him at the first meeting held by this Society which took place in Atlantic City.

William Lintz: We have made experiments with guinea pigs and rabbits, inoculating them every two hours and it was interesting to note the marked polymorphonuclear count that followed, as well as the marked leucocytosis. Leucocytic extract has been prepared by us and we find that we get the best results by the use of distilled water. Out of 15 to 18 cases of pneumonia in which there was a decided leucocytosis, only one case was benefited by the use of the leucocytic extract.

8. CONCERNING ALLERGIC SKIN REACTIONS AS AN INDEX OF IMMUNITY

John A. Kolmer: These experiments were undertaken primarily to determine whether the sera of persons and animals reacting positively and negatively to various allergic skin tests contain lytic antibodies for the corresponding living microorganisms and if so, whether or not these antibodies bear a quantitative relationship to the allergic reactions; secondarily to determine the relationship if any, among bacteriolytic, agglutinating and complement-fixing antibodies in the sera of persons and animals reacting variously to allergic skin tests.

The sera of persons reacting positively and negatively to the typhoidin, luetin and diphtherin tests were studied; also the sera of dogs reacting positively and negatively to the intracutaneous injection of an emulsion of *B. bronchisepticus*.

Bactericidal tests with *B. typhosus*, *B. diphtheria* and *B. bronchisepticus* were conducted with a modified looped-pipet method of Wright; spirocheticidal tests *in vitro* were conducted with a pure culture of *T. pallidum*.

The sera of normal persons possessed a marked bactericidal power for *B. typhosus*; the bacteriolysin for *B. typhosus* in the sera of normal persons and of persons that had typhoid fever or had been immunized with typhoid vaccine was high but bore no relation to the typhoidin skin reactions.

The sera of syphilitic persons in the tertiary stages who reacted positively and negatively to the luetin skin test and the sera of normal persons showed no appreciable spirocheticidal activity for a pure culture of *T. pallidum*.

The sera of persons reacting positively and negatively to the intracutaneous injection of a washed polyvalent antigen of diphtheria bacilli showed an absence of bactericidal power for *B. diphtheria*.

The sera of dogs suffering with distemper; also the sera of healthy dogs and of dogs immunized with *B. bronchisepticus* and reacting posi-

tively and negatively to an intracutaneous allergic reaction were found to be without appreciable bactericidal power for *B. bronchisepticus*.

Agglutinins and complement-fixing antibodies in the sera of persons and animals for these various microorganisms bore no relation to the skin reactions.

These studies demonstrate that there is no experimental support for the theory that allergic skin reactions may be taken as an index to resistance and immunity in so far as it is possible to determine the presence of antibodies *in vitro*.

9. IMMUNITY RESULTS OBTAINED FROM THE USE OF DIPHTHERIA TOXIN-ANTITOXIN MIXTURES AND THE USE OF THE SCHICK TEST

Wm. H. Park and A. Zingher: The authors presented a series of over one thousand cases, that had been actively immunized with diphtheria toxin-antitoxin mixtures. These susceptible individuals were selected by means of the Schick test out of a total of about 10,000 children and adults in ten different institutions.

The mixtures of toxin-antitoxin that were used for immunization were either neutral (66-70 per cent L + to each unit of antitoxin) or slightly toxic (80-90 per cent L + to each unit of antitoxin) to the guinea-pig. The dose was varied from 0.5 cc. to 1.0 cc., and the number of injections from one to three. The injections were made subcutaneously at intervals of 7 days. The local reactions at the site of injection were generally mild; in the older children and adults, the redness and swelling were more marked. General symptoms, like malaise, and temperature of 100-102° F. were noted in 10 to 20 per cent of the cases; in a few the temperature reached 104°F. The symptoms lasted 24 to 48 hours, and then rapidly subsided. Both local and general symptoms were especially evident in those that showed a susceptibility to the protein by giving a combined pseudo and true Schick reaction. No harmful after effects were noted in several thousand injections.

The retests with the Schick reaction showed that only 30 to 40 per cent became immune 3 weeks after the first injection; about 50 per cent at 4 weeks, 70 to 80 per cent at 6 weeks, and 90 to 95 per cent at 8 to 12 weeks. The best results were obtained with the full immunization, consisting of 3 injections of 1.0 cc. each, given at weekly intervals. The duration of the active immunity was studied in a group of children that were followed up for over one and one-half years; these cases showed that the active immunity persisted for at least that length of time. It is possible, that the immunity induced by the injections of toxin-antitoxin starts a continued cellular production of antitoxin, which otherwise would have appeared much later in life.

From their results Park and Zingher concluded that it is advisable to immunize children soon after the first year of life, so as to afford them a protection against diphtheria at a time when the disease is most dangerous. In addition such young children, by not having any hyper-

sensitiveness to the bacillus protein, show very mild local and constitutional symptoms after the injections. An immune child population could thus be developed with the result that fresh clinical cases would be prevented and the bacillus carrier would probably soon disappear as a hygienic factor in our communities.

Interesting and parallel results were noted in guinea-pigs and horses. *Guinea-pigs* are fairly resistant to active immunization with diphtheria toxin-antitoxin, and in that respect they show an almost complete parallelism to the positive Schick cases among human beings. After injections of toxin-antitoxin, an antitoxic immunity develops slowly from the sixth to eighth week. *Horses*, on the other hand, as a rule correspond in their behavior toward small doses of toxin-antitoxin to human beings, who are naturally immune. They both give a ready response, even after a single injection of toxin-antitoxin, and show a distinct increase in the antitoxin content toward the end of the first week. Occasionally, a horse is found that has no antitoxin in the control bleeding; such animals respond slowly to small doses of toxin-antitoxin. It is probable that the tissue cells of the naturally immune human beings and the majority of horses have acquired the property of giving a quick and easy response to the stimulation of diphtheria toxin.

The use of the Schick test in the selection of susceptible children for immunization and in controlling the results of the treatment is justified by the great clinical accuracy which the test has shown during a period of several years in the separation of the susceptible from the immune individuals. The test should be carried out properly with a fresh toxin solution, and the results read daily, for a period of 72 to 96 hours. The pseudo-reactions should be controlled with heated toxin, or recognized by their rapid disappearance after 72 hours. It is only these individuals who give the more marked local reactions after the injections of toxin-antitoxin.

In conclusion Park and Zingher stated that the Research Laboratory of the New York City Department of Health, would supply those who are connected with institutions, and interested in taking up the work, both the toxin for the Schick test, and the toxin-antitoxin for immunization.

10. ANAPHYLACTIC FOOD REACTIONS IN DERMATOLOGY WITH SPECIAL REFERENCE TO ECZEMA

Albert Strickler: Fourteen food products have been tried out in the attempt to demonstrate their relation to various skin diseases. In all, four diseases were studied:—eczema, urticaria, acne and psoriasis. The method of injection employed was the intradermic one. The dose used was $\frac{1}{10}$ cc. In none of our cases was there any manifestation of soreness of the arm or enlargement of the associated lymphatic glands. We studied in all 46 cases of eczema, and the majority of them were kept under observation for some weeks. We studied 10 cases with

urticaria, 13 patients with acne and 11 with psoriasis. From this study we conclude that the anaphylactic skin tests are of value in the etiological diagnosis and in the treatment of various diseases of the skin. These reactions find their greatest value in eczema. In chronic urticaria, acne and psoriasis the tests are disappointing. Approximately 20 per cent of individuals affected with eczema are not sensitive to any of the common foods. Normal people do not react to these tests. As yet, our experience is too limited to draw definite conclusions.

11. COMPARATIVE STUDIES OF THE WASSERMANN AND HECHT-WEINBERG REACTIONS IN SYPHILIS, WITH SPECIAL REFERENCE TO CHOLESTERINIZED ANTIGENS

John A. Kolmer: The Hecht-Weinberg test utilizes the complement and natural antishoop hemolysin of the human serum.

The primary object of this study was to determine whether this test is more delicate than the Wassermann reaction conducted with cholesterolized extracts.

The Hecht-Weinberg tests were conducted after the modification of Gradwohl by which the hemolytic activity and accordingly the proper dose of sheep cells to be used for each serum is determined according to the amount of a 5 per cent emulsion of sheep cells hemolysed by 0.1 cc. of serum.

The same three extracts were used in the Hecht-Weinberg and Wassermann tests; namely, an alcoholic extract of human heart reënforced with 0.4 per cent cholesterol; an alcoholic extract of syphilitic liver and an extract of acetone insoluble lipoids of beef heart. All extracts were titrated for the antilytic and antigenic units in both systems respectively.

With sera collected 24 to 48 hours previously, 93 per cent were found to contain sufficient complement and antishoop hemolysin to permit the conduct of the Hecht-Weinberg test.

In 82 per cent of sera the results of the Hecht-Weinberg and Wassermann reactions were the same.

In 18 per cent of sera the results varied and in this manner: in 15 per cent the Wassermann was negative and the Hecht-Weinberg test positive. Of these reactions the positive Hecht-Weinberg tests were largely correct and occurred mostly with the sera of syphilitic persons under vigorous treatment; in 3 per cent the Wassermann was positive and the Hecht-Weinberg was negative and all of these occurred with the sera of persons in the latent and tertiary stages of syphilis.

With the sera of persons known not to be syphilitic the Hecht-Weinberg test showed about 10 per cent falsely positive reactions; most of these reactions occurred with the alcoholic extract of syphilitic liver and fewest with the extract of acetone insoluble lipoids. All of these sera yielded negative Wassermann reactions with all antigens.

The Hecht-Weinberg test was found unreliable in the diagnosis of syphilis on account of the tendency to yield proteotrophic reactions; it

is more delicate, however, than the Wassermann test and has its greatest value in a negative reaction as a control on treatment with the sera of known syphilitics.

In conducting the Hecht-Weinberg test, alcoholic extracts of syphilitic liver were found least and extracts of acetone insoluble lipoids best suited for this technic.

DISCUSSION OF THE PRECEDING FOUR PAPERS

Edward B. Vedder: I wish to ask Dr. Kolmer whether he has used antigen in a dilution of one to six instead of the customary dilution of one to ten, as recommended by Walker in order to determine whether syphilitics were cured. I would also like to ask if the use of antigen in this quantity (1-6) in the ordinary Wassermann would not furnish as good evidence of cure as that obtained by the use of the Hecht-Weinberg test.

James W. Jobling asked Dr. Kolmer if his failure to obtain bacteriolysis with 0.2 cc. of the serum of vaccinated individuals might not be due to the Neisser-Wechsberg phenomenon?

John A. Kolmer, in replying to Dr. Jobling's question, stated that he did not suspect that complement deviation in the meaning of Neisser and Wechsberg exerted an influence in his technic, because of the progressively higher dilutions of culture until as few as 10 to 100 bacilli per cubic centimeter were obtained.

Oscar Berghausen said that he had been in the habit of using different antigens in determining the Wassermann reaction, a cholesterinized, Noguchi's acetone insoluble fraction, and a luetic liver alcoholic extract. He had been able to obtain a positive reaction with one or the other, or with all, of the antigens, with most of the sera obtained from syphilitic patients. As a rule, when the antigen which had given a positive Wassermann reaction, was employed for the determination of the Hecht-Weinberg reaction, the reaction was again positive. However, using different antigens might result in a positive Wassermann and a negative Hecht-Weinberg; so that a great deal depended upon the selection of the antigen. In his experience, the Wassermann was the control over the Hecht-Weinberg reaction. He placed a great deal of dependence upon the outcome of the reaction with cholesterinized antigens; only occasionally were false positive reactions obtained.

John A. Kolmer, in closing the discussion, said that he had not used Dr. Walker's method with larger doses of cholesterinized antigen in the group of cases mentioned, but that he could well understand that this technic made the test more delicate and was acceptable from this standpoint. He stated that owing to the antilytic properties of cholesterolin he did not employ any of these extracts unless the anticomplementary unit of each was at least twenty times greater than the antigenic unit.

Dr. Kolmer said he overlooked stating that in a study of the non-specific reactions observed with the Hecht-Weinberg technic with nor-

mal sera, that all sera had been likewise subjected to the Wassermann test and all reacted negatively with all antigens including the cholesterinized extracts.

12. THE LOCALIZATION OF A STREPTOCOCCUS IN ANIMALS FROM A CASE OF RECURRING NEURITIS AND MYOSITIS

Edward C. Rosenow (see this volume, page 363).

DISCUSSION

George W. Wheeler stated that streptococci are usually classified, according to their effects on blood-agar plates, as hemolytic or non-hemolytic. The non-hemolytic varieties have been further classified according to their fermentation reactions with different sugars, but wide variations are found by these methods, due to variations in the organisms themselves and to chemical changes in the sugars during the process of sterilizing the media. None of these differential methods gives any clue as to what the streptococci will do when they are in the animal body.

Dr. Rosenow's work begins where these methods end, his original idea being that organisms growing in the human body have certain delicate, transient, biological activities, which are soon lost when the bacteria are grown on artificial media. In order to demonstrate these activities, the organism must be transferred from the human body to animals, the original culture from the patient being used for inoculation, and the lesions in these animals studied. Animal inoculations with recently isolated strains, grown under certain definite conditions, show that the lesions produced in the animals are very often similar to those in the patient from whom the organisms were obtained. Whether this is merely a coincidence or whether it is due to a specific affinity, which the organisms have for certain tissues, can be determined when a great deal of experimental evidence of this kind has been presented and carefully examined. Control animals are necessary in order to rule out the possibility of the lesions being spontaneous.

The speaker said that in work which he had done, according to the methods described by Rosenow, with streptococci from nine cases of arthritis and endocarditis, lesions in joints were found in the animals in 75 per cent—in muscles, 63 per cent; in the heart, 55 per cent, while in other organs lesions were relatively infrequent; appendix, 6 per cent; stomach, 11 per cent; brain and cord, 6 per cent.

Joseph Head: The mouth is such an excellent culture oven that any germ may occasionally be found in it. Obviously only a small percentage of the germs that are introduced into the mouth find lodgment within the tissues and become pathogenic. In making my bacterial examinations for autogenous vaccines, I cauterize the surface of the infected spot with an electric cautery until it turns slightly white and then extract blood from the tissues through the cauterized

spot with a hot platinum spear. Out of 400 such examinations I found streptococci in 95 per cent; *bacillus influenzae* and *micrococcus catarrhalis* in about 75 per cent; while the pneumococcus appeared in about 25 per cent. The pus taken from a pus pocket in one case gave 80 per cent of *pneumococcus mucosus* in the material; the rest contained streptococci, *bacillus influenzae* and *micrococcus catarrhalis*. After giving a vaccine made of these ten times, the mouth healed and the pneumococcus was not discovered again in the various pockets. The work of Rosenow concerning the pulp infection from the circulation is very valuable because it gives a quietus to those who think it an unwise procedure to take out a live infected nerve when it is evident to all thoughtful observers that a living pulp may be a great source of systemic disease.

Oscar Berghausen said he wished to congratulate Dr. Rosenow upon the work he had done. Last summer he had had the opportunity of seeing a case of generalized herpes of the extremities and of the lips, and that he had isolated a definite organism, the *micrococcus pyogenes*. In such cases there was a bad prognosis, most of them dying inside of one or two years. In this case, an autogenous vaccine was administered and was followed by recovery in about two months time. The patient was relieved for a period of eight months, when a slight recurrence took place.

E. C. Rosenow closed the discussion. He said that he preferred using the terms "green-producing" and "hemolyzing streptococci" instead of streptococcus viridans and hemolytic streptococcus, because there appeared to be numerous variants of each strain, particularly of the former, and because one may be transmuted into the other.

He expressed his appreciation of the splendid work of Cole and Dochez and their associates at the Rockefeller Institute, on the classification and immunological observations on pneumococci, and that pneumococci showed in his hands as with these workers fixed characters when grown in the usual way, but that when pneumococci are placed under special environment, they lose their specific agglutinating reactions and take on new features, and they may be converted even into hemolyzing streptococci.

The demonstration of living streptococci in the muscles in the case reported during the quiescent interval is of importance, and in accord with similar findings in ulcer of the stomach, chronic rheumatism and cholecystitis, etc. because it shows that not too much should be expected from the removal of the primary focus in these diseases, which are characterized by exacerbations and quiescent intervals, and serves to explain the nature of these exacerbations and remissions.

13. STUDIES IN THE EPIDEMIOLOGY OF LOBAR PNEUMONIA

A. R. Dochez: A study of pneumococci isolated from individuals suffering from lobar pneumonia has shown that the majority of these organisms fall into definite biological groups. In view of these con-

stant differential characters of the pneumococcus, it has been deemed advisable to study the pneumococci occurring in normal mouths.

It has been commonly assumed that infection in pneumonia is auto-genic and occurs from invasion of the lungs by pneumococci habitually carried in the mouth. If this is so, we should find the same types in the normal mouths which occur during disease. Examination of a series of normal individuals showed this not to be the case. The two types of pneumococcus responsible for the majority of the severe cases of lobar pneumonia are not found in the normal healthy mouth, except in instances where the individual harboring the organism has been in intimate association with a case of lobar pneumonia. When such a condition exists, the organism found in the normal mouth invariably corresponds in type to that found in the lung of the diseased individual.

These studies make it probable that the majority of the cases of pneumonia are dependent either on direct or indirect contact with a previous case.

14. A NOTE CONCERNING THE SPECIFICITY OF PNEUMOCOCCUS TYPES: FATAL INFECTION DUE TO ONE TYPE IN A HORSE PRODUCING AN ANTI-SERUM OF HIGH TITER TO ANOTHER TYPE

A. P. Hitchens, E. K. Tingley and George Hansen: The horse in question had been under treatment for several months with a pneumococcus corresponding in serological reactions with Neufeld Type 1. The last injection was given about one month before death and the bleeding subsequent to this showed that the potency of the serum of this horse was such that 1/100,000 cc. of the serum would protect a mouse against a fatal dose of pneumococci of the homologous type.

Blood culture three days before the death of the horse showed the presence of a pneumococcus not corresponding in type with that with which the animal had been injected. The pneumococcus recovered is still under examination. It does not correspond with Type 2 and is not the mucosus. It does, however, bear strong resemblance to some strains of pneumococci obtained from equine infections. Autopsy of the animal showed pulmonary consolidation and inflammation of the mucous membranes lining the respiratory passages.

DISCUSSION OF THE PRECEDING TWO PAPERS

Edward B. Vedder recommended:

1. Notification of all cases of pneumonia.
2. Prompt visit by a health officer, collection of specimens, and laboratory diagnosis of the type of organism present; (1) in the patient. (2) In the contacts.
3. Isolation of the patient and of any contacts that harbor the type of pneumococcus found in the patient.
4. A negative culture requirement before the patient or carriers are permitted to mingle with the community.

Judson Daland said: The evidence Dr. Rosenow presents proves that in some cases infection of the pulp of a root, which could easily evenuate in peri-apical abscess, may be haemotogenous in origin. Hitherto, most observers have believed that infected root canals and peri-apical abscesses were chiefly due to accidental infection, when these canals are opened in consequence of caries or when they were emptied of their content, and that peri-apical abscesses also occurred by contiguity; i.e., peri-apical abscess in one root infecting another in the immediate neighborhood. This question is of unusual importance because of the proven relationship of peri-apical abscess to many systemic diseases, and lays open to suspicion, not only the roots of capped or devitalized teeth, but also teeth that have shown no caries.

15. STUDIES IN TYPHOID FEVER

A. L. Garbat: Our experiments with reference to the complement fixation test after prophylactic typhoid vaccination may be summarized by stating that a positive complement fixation test after prophylactic typhoid immunization is not a regular occurrence, as it is during or after typhoid fever. This point may be of aid in deciding for or against the diagnosis of typhoid fever in an inoculated individual still having a positive Widal and ill with a suspicious typhoid and negative blood culture. A positive complement fixation test was obtained most often after three injections with a polyvalent vaccine; two injections with this same vaccine or three injections with the single strain vaccine (Rawlings) gave hardly any complement fixation.

In view of the many difficulties in the way of stool examinations in typhoid fever, we have attempted the direct examination of the bile removed from the duodenum. This method has the advantage of avoiding the great number of contaminating bacteria present in the stool; no special media are essential and the bacteria are present in great numbers. We have found that culture of the duodenal contents (bile) removed by means of the duodenal tube seems to be a more reliable and simpler method for the detection of typhoid bacilli than stool examinations.

The serum from convalescent typhoid patients has been employed with distinct benefit in three very severe acute typhoid cases.

DISCUSSION OF DR. GARBAT'S PAPER

A. H. Sinclair: I believe that after the use of typhoid vaccine, if typhoid fever occurs, there must have been some fault either with the technique employed or some fault with the vaccine itself. I do not believe that any patient that gives a positive Widal after typhoid could again contract the disease for many years thereafter. The isolation of the bacillus from the blood or from the stools is certainly superior in those cases recommended for the applicability of the complement fixation test.

A. Zingher said that he had used at the Willard Parker Hospital during the past 18 months, intramuscular injections of fresh whole blood, obtained from convalescent scarlet fever patients. The blood was either citrated or non-citrated. Distinct beneficial effects were noted in toxic cases after injections of blood obtained from patients that were three to four weeks convalescent. In a recent case, a very striking improvement was seen from 300 cc. of fresh blood obtained from a three months convalescent donor. If it should prove that convalescent blood had therapeutic value even as late as 6 months after the disease, then the opportunity for employing the treatment will become much greater. Convalescent blood can be used not only in diseases that result in a more or less permanent immunity, like scarlet fever, typhoid and measles, but also in diseases that are followed by only a short protection, like erysipelas and pneumonia. The blood in these cases should be obtained from donors not more than two to three weeks convalescent. A striking recovery followed in a six year old child, suffering from a severe attack of erysipelas, that he injected with 210 cc. of fresh convalescent blood (7 days).

He has used intramuscular injections of normal blood in late septic cases of scarlet fever. In these the toxemia of scarlet fever was no more in evidence. Injections of normal blood, which can be readily obtained from relatives, are given in quantities of 120 to 240 cc., and repeated every 4 days. Such blood is not given for any specific action, but for its general stimulating and nutritive value, which helps in tiding the patient over a critical period.

Wm. Egbert Robertson said that he had treated a number of patients in the wards of the Episcopal Hospital of Philadelphia, by using subcutaneously, serum obtained from convalescent typhoid and pneumonia patients, which was then administered to individuals at that time ill with the respective diseases. 50 cc. of the serum was given per dose and repeated several times, but the results did not warrant a continuance of the work. Much better results followed in cases of erysipelas where, after raising blisters, he had injected the serum thus obtained, directly into the vein of the same individual or of other individuals ill with erysipelas. Some of these sera were inactivated, while others were not, but as far as cases of typhoid and pneumonia were concerned, no apparent benefit resulted in either case. Dr. Robertson asked the reader of the paper whether his cases were fed by the high calorie method, or whether milk alone was permitted, as these latter are virtually starvation cases, since the majority refuse to take milk in any amount. Dr. Robertson said that it had been his experience with a large number of cases carefully studied bacteriologically by Dr. C. Y. White, pathologist to the Episcopal Hospital, that the well fed cases proved very exceptionally to be ultimate typhoid carriers, while the virtually starved cases or those fed on milk, frequently became carriers. It is nothing more than an assumption, but it seems logical to believe that those well fed developed a greater resistance and a higher antibody formation.

A. L. Garbat closing the discussion, said that all the cases at the German Hospital were placed upon the high calorie method of feeding and the physicians had practically abandoned the starvation treatment. Whether typhoid carriers occurred more frequently in those with the high calorie value or in those under the so-called starvation treatment, he did not know.

May 12, 1916

1. STANDARDIZATION OF ANTIMENINGITIS SERUM BY ANIMAL PROTECTION TESTS

George H. Robinson: (See this volume, page 345).

DISCUSSION OF THE PRECEDING PAPER

Harold L. Amoss believed that a discussion on Dr. Robinson's paper is difficult because of the new method used. One of the most important features of any discussion is the influence towards the standardization of the methods employed. Dr. Robinson used for his experiments a very irregular strain (Isadore) instead of the accepted typical and parameningococcus. Dr. Amoss was glad that the subject of meningitis had been reopened because it is evident that there is much to be learned yet about the preparation and standardization of antimeningitis serum. He reviewed briefly the causes which led to further study of the serum at The Rockefeller Institute.

He called attention to the fact that the ultimate method of testing the value of a serum is by therapeutic application. This has been kept in mind at The Rockefeller Institute where with Dr. Martha Wollstein he has been engaged in the preparation of a polyvalent serum. In other words, particular care has been exercised to corroborate their laboratory tests with the therapeutic tests.

He explained that the animal protection tests have been found to be the least specific of all methods of standardization. He was very much interested in Dr. Robinson's clear cut results in protection tests obtained by diluting the suspension of living meningococci with complement.

Recently he had made some protection tests using young guinea pigs and found that a monovalent antiparameningococcus serum will protect young guinea-pigs against one infective dose of homologous parameningococcus, and against an irregular strain though more serum is required for the latter. The same amount of serum did not protect the animal against a lethal dose of a typical or normal meningococcus. He referred to this experiment to show that in his experience the protection tests roughly parallel the agglutination tests. The complement fixation reaction and opsonization are not as specific as the agglutination reaction. He referred to human cases of meningitis, in which though they were treated with antimeningitis serum, the meningococci

persisted in the spinal fluid. The strains of meningococcus isolated from these cases were not agglutinated in the serum used. When a more polyvalent serum, and one that agglutinates the particular strain was used in treatment, the organisms disappeared, the fever abated, and recovery was uneventful.

On account of the fact that the agglutination tests parallel other tests and are more specific than the latter, agglutination should be used in the standardization of antimeningococcic serum.

He believed that the power to agglutinate in high dilution all known varieties of the meningococcus gives us the best idea as to the value of antimeningitis serum.

James W. Jobling: The existence of parameningococcus strains was not recognized when we began the preparation of the antimeningococcus serum. We realized, however, the necessity of using many strains of organisms. Our results with the complement fixation method were unsatisfactory, therefore it was discarded. According to the work done by Amoss it is possible that some of these results were due to the fact that we were using strains of the parameningococcus. We were not convinced that the death of guinea pigs receiving intraperitoneal injections was due to infection. The clinical picture and the post-mortem findings suggested rather an intoxication. Adopting this idea we attempted to isolate the toxin and use it to standardize the serum, but the results were so unreliable that this method also was discarded.

Harold L. Amoss again referred to the meningitis problem that confronted them. An important point was the great value of time in the administration of antimeningitis serum. It has been observed that many hospitals are casual in their specific treatment of acute infections. He believed that the serologists could assist a great deal in this matter by putting forth every effort to make an early diagnosis, by procuring serum immediately, and by delicately impressing clinicians with the extreme value of early administration.

2. THE CLINICAL SIGNIFICANCE OF THE WASSERMANN TEST

Arthur F. Coca: The Wassermann reaction is a biochemical test: that is, it is performed with reagents whose chemical constitution is practically unknown, some of them being relatively very unstable bodies. The Wassermann "mixture" is subject to considerable variations dependent upon the particular method or modification of the original technic used and also upon the manner of standardizing the different reagents, as well as upon the quality of the antigen preparation available.

On account of the above mentioned technical variations as well as on account of factors heretofore uncontrolled a considerable want of uniformity in the results of the Wassermann test exists. There has yet to be recorded a series of parallel tests carried out by different observers on the same sera in which the results agreed throughout.

As has been pointed out by Uhle and MacKinney, the disagreements

are more common in just the cases in which the need of reliable information is greatest. The results of the Wassermann test are further vitiated by the fact that it is being performed by an ever widening circle of superficially informed and uncontrolled "technicians."

It has been clearly demonstrated that the positive Wassermann reaction is not specific for syphilis. It occurs not only with some regularity in other conditions, but also sporadically in many others.

Analysis of its relations to the therapeutics of syphilis shows that in the great majority of instances the result of the test does not influence the course of specific treatment.

The use of the Wassermann test as a legal criterion of eligibility for marriage must be unconditionally opposed.

3. AN ANALYSIS OF A SERIES OF CASES CHANGING TO WASSERMANN POSITIVE AFTER A WASSERMANN NEGATIVE PERIOD OF TWELVE MONTHS OR OVER

Louis A. Levison: The writer has collected 16 cases fulfilling the conditions mentioned in the title. An analysis of the results of the Wassermann tests in these cases shows that a negative Wassermann, even though obtained over a considerable period of time, cannot be considered as a criterion of cure. It is, however, the best index at the present time of the patient's condition, when considered in conjunction with the absence of clinical manifestations. Carefully considered cases over a much longer period of time will be necessary to determine the final position of the Wassermann as a prognostic agent. Treatment of whatever nature may help to keep the patient Wassermann negative, only to relapse when this is discontinued. In this series all the cases were late or advanced when they received competent treatment. They received mercury either in small and inefficient amounts, some not at all, at the time when treatment could have been of value. Many cases coming under my observation that have been treated intensively from the very start with salvarsan and mercurial injections have gone over one year with negative Wassermanns and this group, while not considered here, stand in sharp contrast to this series of late cases. It may be said then that a long continued negative Wassermann period in a case that has been treated well from the beginning is a much better criterion of cure or permanent arrest than a similar Wassermann negative period occurring in a late or advanced case.

DISCUSSION OF THE PRECEDING TWO PAPERS

Judson Daland stated that he was especially interested in Dr. Coca's paper which gave him the impression that Dr. Coca was somewhat pessimistic regarding the diagnostic value of the Wassermann reaction. Clinicians, however, are fully convinced of the importance and great value of this test, and are most optimistic.

Speaking broadly, he inclined to the opinion that the Wassermann

reaction may be looked upon as having a diagnostic 70 per cent or 80 per cent value, under ordinary conditions; and under special conditions the value of this test is greater. The object to be accomplished by a study of errors of the test is to increase its value; and these errors may be divided into two classes—(1) laboratory and (2) clinical errors. The laboratory errors may be due to the many causes with which the members of this Society are familiar and occasionally may be due to the methods employed or gross carelessness. On the other hand, clinical errors may be due to erroneous belief that syphilis is present or absent, and in such cases when the Wassermann reaction is positive, the error is ascribed to the test. Syphilis may exist and the Wassermann reaction be negative, and one may conclude that the Wassermann reaction was incorrectly performed and should have yielded a positive result. A common clinical error is the failure to recognize the fact that under certain conditions associated with marked disturbances of metabolism, such as uraemia, intestinal toxæmia, fever, etc., occasionally a sero-diagnostic test for syphilis may be strongly positive and syphilis be absent. Obviously such a diagnosis of syphilis solely from the laboratory test, would be a clinical and not a laboratory error.

It is of the utmost importance that this Society should at once take steps to standardize the sero-diagnostic test for syphilis so that the results obtained by the large number of workers may be truly comparable. It is for this reason that I have advocated following the original technique as described by Wassermann, although during the past four years I have modified it by the use of cholesterinized extract of the bovine heart.

From the clinical standpoint, it is of the utmost importance, that all factors concerned in the interpretation of the results of the Wassermann reaction should be given due weight before deducing that a positive or negative Wassermann reaction proves the presence or absence of syphilis.

Oscar Berghausen said that first of all we should be clinicians and be able to interpret the laboratory findings in terms of the clinical picture that was presented. To his mind a great deal depended upon the antigen. A suitable antigen is one that gives positive reactions in luetic cases and negative reactions in non-luetic cases. The results should be reported as either distinctly positive or negative; doubtful reactions having but little real diagnostic value. He believed that a series of antigens, including cholesterinized antigens, should be used in carrying out the reaction. With cholesterinized antigens occasionally false positive reactions are obtained. He had observed such reactions with the blood obtained from patients suffering from advanced malignancy and uremia. Some substance had developed which appeared in the blood current and was able to bind complement in the presence of syphilitic antigens. He claimed that we should be careful in the taking and the preservation of the blood to be examined. Storing of the sera at room temperature, or the delay occasioned by sending the specimen through the mail, might be the cause of false reactions.

4. A STABLE BACTERIAL ANTIGEN WITH SPECIAL REFERENCE TO THE MENINGOCOCCI

George Hansen: (See this volume, p. 355).

5. SERUM REACTIONS FOLLOWING TREATMENT WITH SENSITIZED AND NON-SENSITIZED BACTERIA

G. H. Smith: Work directed toward the determination of the mode of reaction induced by immunization with sensitized and non-sensitized typhoid antigens gave the following results.

1. The agglutinating titer of the sera obtained indicated that the non-sensitized antigen was more efficient. The difference, however, was slight. Agglutinins appeared earlier in the course of the treatment if a non-sensitized antigen was used.

2. Sera produced by treatment with non-sensitized antigen were more active in complement fixation tests.

3. In the case of the opsonic and bacteriotropic indices the differences in values obtained were not of great significance, either in degree or in the rate of production.

4. The degree of leucocytosis produced with the two types of antigen indicated an essential difference. The animals receiving sensitized antigen responded with a greater production of leucocytes, the response after each injection was increasingly greater, and the increase occurred after a shorter interval of time than in the animals receiving non-sensitized antigen.

6. PROPHYLACTIC AND THERAPEUTIC INOCULATIONS IN CERTAIN AFFECTIONS OF THE RESPIRATORY TRACT

George W. Ross, H. K. Detweiler and J. C. Maynard: Reference to the "common cold" was particularly made, first concerning its prevalence and the great discomfort, disability and economic loss which it causes. Its etiology was then discussed. Foster's filterable virus and the *B. rhinitis* (Tunncliffe) and *B. influenzae* (Pfeiffer) were referred to as micro-organisms which in different epidemics seemed capable of initiating the coryza or other early symptoms. It was thought that the secondary invaders (various *streptococci*, *M. catarrhalis*, *pneumococcus*, etc.) probably played an important part and the question was raised as to the likelihood of symbiosis occurring among those organisms, or increasing their parasitism.

In connection with the recent epidemic in Toronto, it was shown that *streptococcus pyogenes* largely prevailed in cultures from the nose and naso-pharynx and sputum. The investigations concerned 38 soldiers of our battalion quartered there. Inasmuch as this battalion's military duties were greatly interfered with as the result of this epidemic, efforts were made to control it, by the use of a vaccine prepared from many strains of the different organisms isolated.

Sixty-two soldiers received from one to six inoculations, twice each week. We were strongly impressed with the results obtained. It is suggested that this method might at least be applied to the control of such epidemics in institutions where a large number of people are closely in contact.

7. THE DIAGNOSIS AND TREATMENT OF SEPTICEMIA

Oscar Berghausen: The term "septicemia" is preferable to "bacteremia." Pyemia is simply a complication of septicemia. We have made blood cultures in 50 cases having symptoms resembling clinically septicemia. Of these, 57 per cent were positive, *streptococcus* being the prevailing organism, although *staphylococcus* was occasionally found, or a bacillus of the colon type in terminal infections. Of 23 patients with a positive blood culture, 74 per cent died and 26 per cent recovered. Of 17 patients with a negative blood culture, 35 per cent died and 65 per cent recovered. This shows the value of the blood culture in suspected cases of septicemia, not only in the diagnosis but also in a prognostic sense. The total white count varied from 7100 to 25,000 in the patients who recovered; from 7000 to 30,000 in those who died. Ordinarily in this series a low white count indicated a bad prognosis. However, this was not invariably the rule. The differential white count is of the greatest importance. When the polymorphonuclear count approaches 90 per cent, particularly when the white count is low, the prognosis becomes grave. In most of these cases the antistreptococcic sera obtainable on the market were employed, before the writer saw the patients; in none was a cure reported. In 13 of the cases autogenous vaccines were employed in addition to the regular symptomatic treatment, and of this number, seven recovered. In true septicemia, the prognosis depends upon the state of health of the individual, the type of organism causing the infection, and the complications that may develop. The longer the course of the disease, the more favorable is the prognosis. These patients should be treated as consumptives; at least they should be given plenty of fresh air and should be fed as liberally as possible without deranging the digestion. The hot pack is indicated in septicemia marked by high temperature and erythema, and in the absence of more serious complications, with stimulation before and after the pack. Digitalis may be employed to enforce the heart's action in the beginning, though we cannot thereby prevent the onset of endocarditis. Of the antipyretics, quinine is the most important and should be used in small doses and often. Apparently good results have been obtained by hypodermoclysis. We believe the continued use of autogenous vaccines is justified. Spinal puncture is indicated when symptoms of meningismus develop.

8. CLINICAL AND PATHOLOGICAL OBSERVATIONS ON THE DANGERS ENCOUNTERED IN CERTAIN TECHNICAL PROCEDURES FREQUENTLY USED BY SEROLOGISTS AND CLINICIANS

H. S. Martland: This paper gave a résumé of the contraindications, dangers and accidents met with in lumbar puncture, intraspinal and intracranial injections, intravenous medications, blood transfusions, etc. The observations of the author were based on clinical and pathological data, from over 1500 autopsies at the Newark City Hospital. The paper included the presentation of pathological specimens.

9. TREATMENT OF TUBERCULOSIS PULMONALIS BY TUBERCULIN

A. N. Sinclair: Most notable proof of the value of tuberculin is seen in the difference in the results obtained at Leahi Home before and since the adoption of tuberculin treatment. No attempt has been made to classify the cases because as soon as classification is attempted, considerable opportunity for error is offered. Out of 309 cases before the period tuberculin was used, 27.2 per cent of all cases treated were either arrested or were able to return to their former occupations, while out of 506 cases treated after tuberculin was adopted, 50.1 per cent were enabled to resume their former occupations. Another proof of the value of tuberculin is the almost immediate and continuous improvement that has occurred in many cases that did not receive treatment at Leahi Home but who were given tuberculin treatment. Patients having suspicious signs of tuberculosis but in whom the diagnosis lacks confirmation, offer golden opportunities for the administration of tuberculin; there is no valid argument against its use in the nontuberculous. In such cases not only does tuberculin afford a means of diagnosis from observation of the reaction to its injection, but as I have invariably noted, the patients at once begin to put on weight, gaining from three to six pounds in the first month of treatment; these cases in which tuberculin treatment is begun so early that diagnosis is questionable, seldom, if ever, fail to be restored to health. Failure in the use of tuberculin may rest on three factors; on the tuberculin itself; on the dosage, and on the selection of unfavorable cases. The dosage should be regulated, not by the "rule on the bottle method" but by the immunizing method. The production of antibodies to the tubercle bacilli is accomplished more safely and just as certainly by continued small doses as by large ones. I never go above 1/300 and rarely above 1/500 mgm. As long as a patient is steadily improving, there is no need to increase the dose. The great danger in using tuberculin is that of producing too great focal reaction. We have had no means of gauging this—it is suggested that the albumin content of the sputum be adopted for this purpose—the amount of albumin indicating the amount of destruction of lung tissue.

Increasing albumin content means the dose is too large, or it should be discontinued—a decreasing content and no improvement means the dose is too small.

Of course this index of dosage must be considered in conjunction with other indications.

10. THE VALUE OF TUBERCULIN IN TREATMENT OF TUBERCULOSIS LYMPHNODEITIS

George P. Sanborn: An attempt was made at evaluation of tuberculin in a series of 58 cases of tuberculous lymphnoditis. Gradually increasing doses of bacillus emulsion were given. Constitutional reaction was avoided so far as possible. The patient's condition of living was practically the same during tuberculin treatment as during the period when the lymph nodes were developing. Certain cases obviously requiring improvement in hygienic conditions, such as absolute rest, improvement in diet, and so on, are excluded from this report. In 20 cases the patients have been seen at periods of from two or three months to several years after the cessation of tuberculin treatment; in the rest, results were noted soon after tuberculin treatment was stopped. By excluding, then, other therapeutic means, it is believed that the results furnish a more accurate index of the efficiency of tuberculin than otherwise would have been possible.

11. MALIGNANT COURSE IN DIPHTHERIA; EXTENSIVE RECURRENCE OF MEMBRANE DESPITE LIBERAL DOSES OF ANTITOXIN, ESPECIALLY IN LATE TREATED CASES

G. Huestis Fonde: Probably most physicians of wide experience have observed cases of diphtheria in which seemingly adequate doses of antitoxin resulted in prompt exfoliation of membrane, subsidence of pulse rate and temperature approaching normal, followed by a quick regrowth of membrane more rapid and extensive than before, accompanied by sudden return of toxemia, shock, etc. The same symptom complex may be repeated again and again. Such a course generally applies to late treated cases, or cases receiving too timid use of antitoxin at the initial dose; but in several instances the writer has witnessed such a course in cases treated early even when the initial dose was large as compared with the usual recommendations of standard authorities. There is need of establishing a reasonably defined margin beyond which parenteral introduction of a foreign serum may not be given, and also need of a margin beyond which trikresol or other preservatives in sera may not be given. I would suggest the intraperitoneal injection of antitoxin after the method employed in laboratory animals. Any amount of serum may be given in this way and the result should be rapid and steady absorption. The case I am reporting was a late treated case in desperate clinical need. After administering 150,000 units of antitoxin intramuscularly and subcutaneously, on the seventh day of the disease as a last heroic measure 410,000 units were injected intraperitoneally within six hours time through a rubber catheter.

There followed a free and thorough exfoliation of the membrane, which when it loosened up was found to involve the larynx, trachea, and bronchial tubes deep into the lungs, and also the post nares and pharynx. The patient died in a syncopic attack following the exhaustion incident to a choking paroxysm due to the large mass of tough membrane in the larynx. The writer believed that the results obtained so late in this case justified the conclusion that the patient would have been given an excellent chance of recovery if the massive dosing intra-abdominally had been employed when he first reached the case on the fourth day, and that major dosing in this manner had an important field of application in cases not to be met by one full dose intravenously. He also believed the percentage of fatal cases of diphtheria might by this means be safely reduced.

PRESIDENT'S ADDRESS

THE RELATION OF LIPOIDS TO IMMUNE REACTIONS

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The relation lipoids bear to immune reactions has been studied extensively during the past few years, but in this review it will be impossible to give more than a brief summary of the work which has been done.

The relation of lipoids to biological phenomena did not receive much consideration until Meyer and Overton suggested their possible importance in narcosis. According to these authors the cell wall is composed chiefly of lipoids, a view that has been held for plant cells for a number of years. If this view is accepted we must concede the possible importance of lipoids in protecting bacteria and the cells of the body against antagonistic substances.

RESISTANCE OF BACTERIA TO INJURIOUS AGENTS

Petersen and I (1) have shown that bacteria are protected from the action of ferments by the unsaturated fatty acid compounds present in the cell, and that oxidizing agents such as iodine, hydrogen peroxide, etc., will destroy this protective action.

It has been known for some time that many oxidizing agents have a bactericidal action. Arkin (2) states that sodium iodoxybenzoate, an organic peroxide, stimulates the production of hemolysins and agglutinins in rabbits if injected intravenously after immunization. He also observed that the same substance stimulates phagocytosis.

Treatment of bacteria with thermostable immune bodies also renders them more susceptible to the action of ferments, and experiments

which we are now conducting suggest that these substances act in a manner somewhat similar to that of oxidizing agents.

It must be borne in mind that bacteriolysis is almost never obtained with undiluted immune serum, while bacteriolysis *in vivo*, except in the peritoneal cavity, has not been demonstrated. These facts may be explained by our observation that immune sera in the proper dilutions—dilutions with which bacteriolysis is obtained—render the specific bacteria much more susceptible to the action of ferments, whereas bacteria treated with the undiluted serum—dilutions not giving bacteriolysis—are rendered much more resistant. In the latter case it was found that the bacteria had absorbed the antiferments of the serum. This, we believe, explains the Neisser-Wechsberg phenomenon.

Hailer and Rimpau (3) found that lipid soluble substances are bactericidal, and that a definite relation exists between this action and the lipid solubility, narcotizing action and the bactericidal action of the substances tested.

INFLUENCE OF ENVIRONMENT

Bacteria may be so situated as to become inaccessible to the agents that normally bring about their destruction. This is particularly true in tuberculosis and syphilis where the necrotic and avascular tissues surrounding a focus may be such as to prevent the entrance of immune substances, or other bactericidal agents. In such instances it is necessary to break down the barriers protecting the organisms before determining the value of immune sera or chemotherapeutic substances.

Our demonstration (4) that the failure of autolysis is due to the protective lipoids makes this phase of the subject more accessible to attack. In addition, we have shown that oxidizing agents such as iodine inhibit the action of the antiferments, accelerate autolysis, and as a result, expose the infecting organisms to attack from without.

ANTIGENS

Froin (5) found that lipid free corpuscles injected into animals produce agglutinins, but no hemolysins, whereas the lipoids produce lysins. Bang and Forssman (6), in similar experiments, obtained complement fixation with the immune sera, using the lipoids of the homologous cells as antigens. Dautwitz and Landsteiner (7) obtained similar results, but Kurt Meyer (8) was unable to confirm these observations. Meyer concludes that the antigens used by these authors were impure because they contained proteins. This author found, however, that the sera of rabbits inoculated with watery extracts of tape-worms possess specific precipitins and specific complement-binding substances for the lipoids of the tape-worm.

Thiele and Embleton (9) were unable to show that lipoids, except those obtained from tape-worms and from tubercle bacilli, would act as antigens. These authors found that lipoids obtained from sheep's

corpuscles and from various tissues of the cat did not act as antigens, whereas the lipid free cells still possess the property of causing the production of the various immune bodies when injected into rabbits. However, they found that extracts obtained by treating fresh tissue with 85 per cent alcohol were active. In preparing the lipoids used in their work the tissues were first dried and then extracted with absolute alcohol. Thiele and Embleton believe that the results obtained with lipoids extracted from fresh tissues were probably due to the fact that proteins were also present, and that this explanation probably holds true for the contradictory results obtained by others with lipid antigens. They also found that unextracted tubercle bacilli produce powerful precipitins against the tubercle phosphatids, while animals immunized against tubercle phosphatids develop precipitins against these substances.

Nicolle (10) and Pick (11) believe that the precipitable substances in antigens are soluble in alcohol.

Much (12) was able to obtain positive complement fixation tests with the sera of patients suffering with infections due to acid fast micro-organisms, using nastin as antigen, while Gottlieb and Leffmann (13) were able to prepare specific antibodies (hemolysins) by treating animals with ethereal extracts of red blood corpuscles.

The differences in results obtained by the various investigators suggest that lipoids in certain combinations may act as antigens, while the pure lipoids have not this property. This may explain why the protein free lipoids of tape-worms and tubercle bacilli act in this manner.

PHAGOCYTOSIS

Stuber (14), Dewey and Nuzum (15) and others have shown that certain lipoids, chiefly cholesterol, inhibit phagocytosis. Stuber obtained similar results with the cholesterol esters of oleic and palmitic acids. According to Dewey and Nuzum the leucocytes of the blood of animals inoculated with cholesterol are less phagocytic for bacteria than the corpuscles of the same animal previous to inoculation.

Graham (16) observed that the opsonins were markedly decreased in the serum of patients following ether anesthesia. A similar decrease in opsonins was noted when serum was treated with ether, but this inhibiting action was lost when the ether was removed by passing a current of air through the serum. This suggests that the inhibition of phagocytosis is not due to injury of the cells. In subsequent experiments he found that rectal injections of olive oil prevented this decrease to a large degree. Lecithin injected subcutaneously had a similar action.

Müller (18) states that the lipoids obtained from typhoid bacilli mixed with immune sera does not increase or decrease phagocytosis of the untreated bacilli. He concludes that bacterial lipoids are unimportant in the process of phagocytosis.

AGGLUTININS

According to Stuber (19) the serum of rabbits that receive subcutaneous injections of fat free typhoid bacilli has a low agglutinating value, while the agglutinating value of the sera of animals injected with mixtures of the lipoids and lipid free bacilli, is high. He believes that the agglutinins are produced as a result of the stimulus afforded by the fats liberated after destruction of the bacteria.

Stuber also found that immune serum extracted with ether loses most of its agglutinating power, and that normal serum to which the ether extract is added acquires an agglutinating value almost equal to that of the immune serum from which the extracts were obtained. The serum of normal animals that receive intravenous injections of extracts of the immune serum also contain strong agglutinins. Graham (20) states that ether anesthesia does not effect the agglutination titer of sera.

HEMOLYSINS

Neuberg and Rosenberg (21) believe that hemolysins are lipolytic, while Noguchi (22) has suggested that some of the serum and cellular complements may be salts of the higher fatty acids with weak organic bases. Hektoen and Ruediger (23) observed that calcium, barium and strontium ions inactivate complement, and, as Wells (24) has pointed out, these substances precipitate fatty acids.

Friedemann and Herzfeld (25) believe that complement is in no way associated with lipoids. They extracted sera dried on filter paper for one hour with certain solvents, and found that the complement was still active when the sera were dissolved in water. Surányi (26), however, demonstrated that fully 25 per cent of the lipoids of the serum were not extracted by this method, and believes that the conclusions arrived at by Friedemann and Herzfeld were unjustified. Kurt Meyer (27) states that the hemolytic immune bodies are not lipoids as they cannot be extracted with lipid solvents; the sera are just as active after extraction.

Neuberg and Reicher (28) and others have demonstrated a marked parallelism between agglutinative, hemolytic and lipolytic substances.

Bull and I (29) demonstrated what we believed to be immune lipases in hemolytic sera, but we were unable to show that they were essential for hemolysis.

Thiele and Embleton (30) failed to demonstrate the presence of specific lipolytic ferments in hemolytic sera, and state that the sera of guinea pigs inoculated with the phosphatids of sheeps red cells did not contain hemolysins.

ANAPHYLAXIS

Bogomolez (31) states that animals sensitized with lipid free egg-yolk will not give an anaphylactic reaction when reinoculated with lipid free egg-yolk, or with the extracted lipoids. Thiele and Emble-

ton (32) however, found that lipid free egg-yolk is able to sensitize and cause anaphylactic shock. According to their results, the lipoids are not able to sensitize, but they do cause shock. Wilson (33), also, was unable to sensitize animals with lipoids. Thiele and Embleton apparently failed to take into consideration the fact that lipoids when given intravenously may cause symptoms in unsensitized animals similar to those seen in anaphylactic shock.

Saula (34) states that there is a marked increase in soaps, fatty acids and lipoids, subsequent to sensitization. The increase begins on the fifteenth day after sensitization, and increases to the twentieth day. There is then a gradual decline, the serum containing the normal amount of these substances on the forty-fifth day. He suggests that anaphylaxis may be due to increased saponification.

In this respect it is interesting to note that Petersen and I (35) found that soaps injected intravenously into guinea pigs in proper doses produce the symptoms and postmortem findings of anaphylaxis.

Thiele and Embleton (32) found that animals inoculated with protein-free phosphatids of tubercle bacilli react with their specific antigens, but only slightly with emulsions of the whole tubercle bacilli. Intravascular clotting was frequently noted in the animals that died. These authors were also able to produce passive sensitization with the sera of the sensitized animals.

It has been known for some time that the serum antitrypsin is increased following the recovery from anaphylactic shock. With this in mind Petersen and I (35) investigated, first, the action of lipoids when given with the intoxicating dose of antigen, and secondly, the influence of increasing the antitryptic power of the serum. Briefly, we found that increasing the antitryptic power of the serum and the addition of soaps to the intoxicating dose, enabled the animal to resist several times the amount of the specific protein fatal for the controls, while a smaller dose of the antigen was required when lipid free proteins were used.

SEROTOXINS AND ANAPHYLATOXINS

The toxic substances obtained by treating sera with bacteria are more generally known under the name of anaphylatoxins. At first it was thought that these toxins were derived from bacteria, but Friedemann (36), Keysser and Wassermann (37), Ströbel (38), Bordet (39), and other investigators, obtained toxins, that acted in a similar manner by treating sera with kaolin, agar, starch, etc.

Petersen and I (40) have shown that the removal of the lipoidal anti-ferments from serum permits the formation of toxic substances, which we have termed "serotoxins." These toxic substances are formed through the action of the serum proteases on the serum proteins as soon as the protective lipoidal substances are removed. In our experiments we found that while there was no loss of nitrogen from bacteria treated in this manner, there was absorption of serum-antiferment from the serum, and accompanying this loss of antiferment power, a pro-

portionate increase in toxicity. Other experiments showed that the lipoidal substances had been adsorbed by the bacteria, which now became much more resistant to such ferments as trypsin.

It may be that similar toxic substances are formed *in vivo* in some of the bacteremias, for instance, in anthrax. Here there is an enormous number of bacteria in the circulating blood, and we have already shown that *in vitro* toxic substances, independent of those obtained from the bacteria, are formed under these conditions.

ACTION OF BACTERIAL TOXINS

Landsteiner and Botteri (41) found that 0.001 grams of protogen neutralized one lethal dose of tetanus toxin, and that palmitic and stearic acids also neutralize tetanus toxin. Takaki (42) studied the lipoids of human brains and found that the active constituents were the cerebroside. Cerebrinic acid was particularly active; one gram adsorbed sufficient tetanus toxin to kill 1200 mice. These results were confirmed by Loewe (43). According to Laroche and Grigaut (44), however, the toxin is bound only by phosphorus containing lipoids as kepalin and lecithin, and not by the phosphorus free lipoids as cerebrin.

Raubitschek and Russ (45) observed that 1 per cent solutions of sodium oleate neutralize diphtheria and tetanus toxins. The soap is more active than the pure oleic acid. He believes that it is a physico-chemical reaction, and that the greater activity of the soap is due to its greater solubility. The soap is inactive in the presence of serum and gelatin.

De Waele (46) found that small amounts of lecithin activate toxins, while large amounts inactivate.

COMPLEMENT DEVIATION

Bergel (47) believes that a positive Wassermann reaction is due to the presence of lipases which are produced as a result of the antigenic properties of "lueslipoid," and that a positive reaction is obtained only when the lymphocytes in the circulating blood are increased in number. The fact that the antigens used in the Wassermann reaction are lipoids lends some support to this hypothesis. However, it must be remembered that lipid solvents such as alcohol, ether, chloroform, etc., given to patients presenting positive Wassermann reactions frequently cause their sera to react in a negative manner, while it has also been observed that sera of normal individuals sometimes give positive Wassermann reactions following chloroform narcosis. From this it may be seen that alterations in the lipid content of the serum have an important bearing on the reaction, a conclusion borne out by the work of Kolmer and Pearce (49).

These results would not necessarily destroy the hypothesis of Bergel that the reaction is due to lipase action, or rather, esterase action, as variations in the concentration of the substrate may have an important influence on the action of ferments.

It is well to remember that soaps—especially those of the unsaturated fatty acids—are anticomplementary in most minute amounts, so that a combination of the alkalies of the blood with the acids liberated by the esterases, may afford sufficient amounts of these substances to bring about a destruction of the complement. An alteration in the state of dispersion of the colloids of the serum owing to the increased acidity might also explain the reaction. This is suggested by the results obtained through changing the isotonicity of the serum by dialysis, etc.

Bergel (48), and more recently, Kolmer (50), found that the serum of normal animals inoculated with lecithin solutions binds complement in the presence of lipoidal antigens with increased avidity. The sera of normal animals may bind complement in the presence of lipoidal antigens, but according to Kolmer this property may be partly removed by treating the sera with ether or chloroform. Kolmer and Pearce (49) also found that the administration of ether and chloroform to animals caused the previously positive sera to react in a negative manner, but that the reaction was not changed in animals receiving urethan—a non-solvent for lipoids.

Landsteiner and v. Eisler (51), Bang and Forssmann (52), Noguchi (53) and Kolmer (50), believe that the anticomplementary substances found in normal sera are chiefly lipoidal in nature. Zinsser and Johnson (54) however, were unable to extract the thermolabile anticomplementary substances, with lipoid solvents.

Citron and Reicher (55), Peritz (56) and others maintain that the lipase content of the serum is considerably increased in leucetemics. The same is said to be true of patients afflicted with leprosy, tuberculosis and other diseases, the causative organisms of which have large amounts of lipoids. Eckstein (57) claims that syphilitic sera giving marked Wassermann reactions have a lipolytic action on fats after inactivation, whereas the fresh sera have no such action.

According to Thiele (58) the antigenic property of phosphatids is not due to the nature or variety of phosphatids used, but depends upon a substance that is present as a result of autolytic degradation of the tissue, and which is brought out with the phosphatids.

ABDERHALDEN REACTION

Since the Abderhalden method of dialysis has been available for clinical purposes, numerous reports of results, both favorable and unfavorable, have been published. It was soon found that positive reactions could be obtained with other substances beside placental tissue, and in other conditions besides pregnancy. Plaut (59) found that with guinea pig serum positive reactions were obtained with kaolin, kieselsol, barium sulphate, etc. These results have been confirmed by several investigators, and show that digested products may be derived from the serum and not from the added antigen.

In discussing the formation of toxic substances by treating sera

with bacteria, kaolin, starch, etc., it was stated that the formation of these toxins is due to the action of the proteases on the serum proteins following the removal of the antiferments. The same explanation holds good for the Abderhalden reaction (60). In this test the placental tissue added to the serum adsorbs the lipoids constituting the antiferments and thus permits the non-specific proteases—which are increased in pregnancy, to act upon the unprotected serum proteins. Similar results may be obtained in any condition accompanied with increase of serum proteases as for instance pneumonia certain cachexias, certain stages of tuberculosis, etc. Other substances such as kaolin, starch, etc., may replace the placental tissue, though they are not usually so efficient. Similar observations have been made by Bronfenbrenner (61), Falls (62), and other observers.

RELATION OF LIPOID SOLVENTS TO GENERAL INFECTIONS

According to Rubin (63) the hypodermic injections of alcohol, ether and chloroform reduce the leucocyte count and render the animals more susceptible to the inoculation of pneumococci and streptococci. Laitinier (64), Delearde (65) and Abbott (66) also found that alcohol made animals more susceptible to infections, while Zimin (67) observed that ether and chloroform narcosis make animals less resistant to proteus vulgaris.

Until recent years the entire focus of attention of immunologists centered about proteins and the chemistry of proteins. This is natural, considering the remarkable development of protein chemistry that occurred during the time when immunology was in its infancy. At present, however, we have come to realize that the lipoids of the body are of far reaching consequence in almost all vital cellular phenomena, and to the realization of this fact the work of Funk has contributed materially. When contrasted with the relatively inactive protein molecule the lipoids and fats stand out particularly by their great reactivity both physically and chemically. The rapidity with which colloidal changes can occur, the ease with which the water insoluble forms may saponify and become water soluble, the intersolution of one form in the other, the frequent occurrence of combinations of proteins and lipoids, are all factors that tend to explain the constantly manifest influence of the lipoids in the various immunological and physiological balances. And to these we must add the amazing latitude that is found in both the quantitative and the qualitative variations following certain physiological and pathological changes.

These considerations indicate that the fats and lipoids may play an important rôle in at least some of the immunity reactions.

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A CUTANEOUS REACTION IN CANINE DISTEMPER

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The etiology of canine distemper, one of the most widespread and fatal of the infectious diseases among dogs, has been, since 1874, the subject of numerous investigations, and various microorganisms and a filterable virus have been assigned as the cause. In 1910 Ferry (1) described a bacillus apparently distinct from any previously isolated or described, which he claimed was the primary and essential etiological factor in canine distemper. In 1911 Ferry (2) claimed to have experimentally inoculated puppies with pure cultures of this bacillus under rigorous precautions against accidental contact infection, and to have successfully reproduced the disease with the classical symptoms and lesions of distemper, such as nasal discharge, conjunctivitis, bronchitis and broncho-pneumonia, cough, emaciation and pustular eruption of the skin. Since then Ferry (3, 4) has reported that this bacillus may be the cause of a destructive epizootic among other laboratory animals such as guinea-pigs, rabbits and monkeys and proposed the name of *Bacillus bronchisepticus* for the microorganism. Quite independently of Ferry this same bacillus was isolated by McGowan (5) from cases of distemper among dogs, cats, guinea-pigs, rabbits, a goat, a monkey, two ferrets and from the nose of a laboratory assistant, who was constantly handling animals and had suffered for over a year with a severe nasal catarrh. Torrey and Rahe (6) after making extensive researches in this disease, conclude that *B. bronchisepticus* (Ferry-McGowan) is the essential primary etiological factor in canine distemper.

The present communication is part of an investigation in canine distemper that has been conducted during the past two

years in the animal house of the medical school of the University of Pennsylvania. A full report of the results of certain bacteriological and immunological studies in this disease will be given later; the object of this paper is to record the results of anaphylactic skin and ophthalmic tests with a polyvalent antigen of *B. bronchisepticus*, for which we propose the name *bronchisepticin*, among dogs sick with distemper. Some of the animals are known to have had the disease while under our own observation and others were healthy and apparently normal at the time of these tests, although nothing was known or could be learned of their previous history. A large percentage of dogs and particularly of those having distemper or known to have had the disease, yielded well defined skin reactions; a much smaller number reacted to the conjunctival instillation.

PREPARATION OF BRONCHISEPTICIN

The antigen with which this work was conducted was prepared as follows: Three strains of *B. bronchisepticus* received from Dr. Torrey in 1912 and 1913 and two strains isolated by one of us (Harkins) from dogs with distemper, were grown in Blake bottles of neutral glycerine agar for seventy-two hours; at the end of this time the growths were removed with sterile physiological salt solution, heated at 60°C. for one hour and thoroughly centrifugalized. After being washed once more with salt solution in the same manner the emulsion was shaken mechanically, filtered through four thicknesses of sterile cheese cloth, cultured for sterility and preserved with 0.5 per cent phenol. The resulting emulsion was of a yellowish color, perfectly homogeneous and it contained about ten billion bacilli per cubic centimeter.

METHOD OF APPLICATION

The skin tests were conducted by injecting intracutaneously with a sterile syringe and fine needle (No. 26), 0.1 cc. of the emulsion into the skin of the inner portion of the thigh or lower abdomen. The eye tests were conducted by instilling a drop of the same emulsion into the lower conjunctival cul-de-sac of one eye.

Both the skin and eye tests were recorded forty-eight hours after inoculation.

THE REACTIONS

Skin tests. In a number of animals no reaction at all was apparent or but a very small area of erythema about the needle puncture which was disregarded as being purely traumatic.

Positive reactions were of two kinds: papular and pustular. Both were characterized by areas of erythema of varying size and marked edema. In the majority of instances the area of erythema could be measured and the size of each reaction is therefore given in the respective tables. The pustular reactions were usually more severe than the papular variety and presented at the end of the second day after inoculation a wide area of erythema with a central papule bearing a bluish-yellow cap of beginning pustulation. Although the temperatures of animals were taken daily no distinct evidences of general reactions could be detected.

Eye tests. In the majority of instances there was no apparent reaction. Occasionally a dog reacted with a mild conjunctivitis characterized by erythema and purulent discharge, but throughout the work there was a notable absence of conjunctival reactions among all classes of dogs.

RESULTS OF THE TESTS

a. In dogs showing no evidences of distemper at the time of these tests. The results of the skin and ophthalmic tests in a group of apparently normal dogs is shown in table 1.

These animals were brought to the animal house with absolutely no information regarding their previous medical history and therefore no statement can be made as to whether or not they had had distemper at an earlier period of life. Since distemper is so common particularly among young dogs, constituting a disease about as prevalent among young dogs as measles is among children, it is practically certain that some of these animals had had the disease. After admission every animal was kept under close and daily observation and in the table we

TABLE 1

Results of bronchisepticin tests in dogs showing no evidences of distemper at the time of tests

NO.	APPROXIMATE AGE	CLINICAL HISTORY*	ANAPHYLACTIC TESTS	
			Skin	Eye
			<i>cm.</i>	
875	1 yr.	Normal 3 wks.	3.0×3.0	—
859	1 yr.	Normal 3 wks.	0.6×0.8	+
9083	2 yrs.	Normal 2 wks.	—	—
692	1 yr.	Normal 10 wks.	—	—
9084	3 mo.	Normal 3 wks.	—	—
9086	3 mo.	Normal 2 wks.	0.7×0.8	—
9089	3 mo.	Normal 2 wks.	—	—
878	10 mo.	Normal 2 wks.	2.0×3.0	—
880	1½ yr.	Normal 10 days	0.6×0.8	—
883	1 yr.	Normal 1 wk.	—	—
885	10 mo.	Normal 1 wk.	—	—
884	10 mo.	Normal 1 wk.	—	—
886	10 mo.	Normal 1 wk.	—	—
712	1 yr.	Normal 10 wks.	—	—
892	8 mo.	Normal 1 wk.	—	—
889	6 mo.	Normal 1 wk.	—	—
871	8 mo.	Normal 3 wks.	1.0×0.8	—
828	1 yr.	Normal 1 mo.	1.0×1.0	—
888	1 yr.	Normal 1 wk.	—	—
893	10 mo.	Normal 1 wk.	—	—
873	1 yr.	Normal 2 wks.	—	—
787	10 mo.	Normal 6 wks.	—	—
822	1 yr.	Normal 5 wks.	—	—
894	1 yr.	Normal 1 da.	0.6×1.2	—
853	1 yr.	Normal 4 wks.	1.0×1.0	—
948	4 mo.	Normal 1½ mo.	—	—
906	1 yr.	Normal 2 mo.	1.0×1.5	—
747	2 yr.	Normal 4 mo.	—	—
568	1½ yr.	Normal 7 mo.	0.5×0.5	—
858		Normal 1 wk.	—	—
933	1 yr.	Normal 1½ mo.	0.6×0.8	—
925	1 yr.	Normal 2 mo.	—	—
923	1 yr.	Normal 2 mo.	0.5×0.5	—
941	1 yr.	Normal 1½ mo.	—	—
710	2 yr.	Normal 4½ mo.	—	—
962	1½ yr.	Normal 1½ mo.	—	—
627	2 yr.	Normal 6½ mo.	1.4×1.0	—

* The periods of time noted in the table are those during which the respective dogs were in the animal house under our direct observation.

have given the period of observation of each dog in the animal house before the tests were applied.

Of the twenty-seven dogs in this group, fourteen (or 35.9 per cent) yielded positive skin reactions and one an ophthalmic reaction.

b. In dogs sick with distemper. The results of the tests in nine dogs presenting evidences of distemper are shown in table 2.

TABLE 2
Results of bronchisepticin tests in dogs suffering with distemper

NO.	APPROXIMATE AGE	CLINICAL HISTORY		ANAPHYLACTIC TESTS	
		Previous history	Condition at time of tests	Skin	Eye
				cm.	
681	6 mo.	Distemper 10 wks.	Distemper	0.8×0.8	—
882	6 mo.	Distemper 10 da.	Distemper	0.5×0.5	—
861	1 yr.	Distemper 1 mo.	Distemper	1.0×2.0	—
739	6 mo.	Distemper 2 mo.	Distemper	—	—
891	10 mo.	Distemper 1 wk.	Distemper	—	+
869	10 mo.	Distemper 3 wks.	Distemper	1.0×2.0	—
682	8 mo.	Distemper 3 mo.	Distemper	1.0×1.5	++++
969	6 mo.	Distemper 1 mo.	Distemper	0.6×0.6	—
927	6 mo.	Distemper 6 mo.	Distemper	—	—

— = negative reaction; + = mild reaction; ++++ = severe reaction.

As shown in this table the duration of the infection varied from a week to as long as six months and positive skin reactions were observed in 66.6 per cent. In one instance (891) a mild ophthalmic reaction was observed while the skin reaction was of such mild character that we recorded the result as negative.

c. In dogs known to have had distemper. The results of tests in twenty dogs received in the animal house in apparently normal and good condition, all of which developed distemper and recovered, are shown in table 3.

All of these animals had had the disease, but usually of a mild character, as those severely infected either had succumbed or were destroyed. The time elapsing between recovery and the application of the tests is shown in the table.

TABLE 3

Results of bronchisepticin tests in dogs known to have had distemper

NO.	APPROXIMATE AGE	CLINICAL HISTORY		ANAPHYLACTIC TESTS	
		Previous history	Condition at time of tests	Skin	Eye
				cm.	
750	10 mo.	Distemper 2 mo. ago	Normal	1.0×1.4	—
718	1 yr.	Distemper 9 wks. ago	Normal	—	—
555	7 mo.	Distemper 5 mo. ago	Normal	1.0×1.0	—
842	1 yr.	Distemper 1 mo. ago	Normal	1.5×1.5	—
846	10 mo.	Distemper 1 mo. ago	Normal	1.0×1.0	—
809	1 yr.	Distemper 6 wks. ago	Normal	0.5×0.5	—
829	1 yr.	Distemper 1 mo. ago.	Normal	—	—
874	1 yr.	Distemper 2 wks. ago	Recovered	0.5×0.8	—
862	10 mo.	Distemper 3 wks. ago	Recovered	1.0×1.2	—
801	10 mo.	Distemper 6 wks. ago	Recovered	1.5×1.2	—
865	9 mo.	Distemper 3 wks. ago	Recovered	—	—
766	1 yr.	Distemper 2 mo. ago	Recovered	—	—
944	1 yr.	Distemper 1½ mo. ago	Recovered	—	—
960	4 mo.	Distemper 1½ mo. ago	Recovered	—	—
957	4 mo.	Distemper 1½ mo. ago	Recovered	—	—
685	1½ yr.	Distemper 5 mo.	Recovered	—	—
857		Distemper 3 mo. ago	Recovered	1.0×1.5	—
778	1 yr.	Distemper 4 mo. ago	Recovered	1.2×1.0	—
938	1 yr.	Distemper 1½ mo. ago	Recovered	1.0×1.0	—
682	1 yr.	Distemper 6 mo. ago	Recovered	1.5×1.0	—

Among these animals positive reactions were observed in twelve (or 60 per cent); the longest interval following recovery was six months (682) and this animal presented a positive reaction. It is probable that sensitization persists for much longer periods of time as in glanders and tuberculosis.

d. In dogs after active and passive immunization. At the time these tests were made we had a special group of dogs under observation, all of which had received three injections of a vaccine of *B. bronchisepticus* at intervals of a week, to test the value of this vaccine from the standpoint of active immunization; also a group of dogs suffering with distemper and receiving subcutaneous, intramuscular and intravenous injections of anti-bronchisepticus (horse) serum. The results of the tests in these dogs are shown in table 4.

TABLE 4

Results of bronchisepticin tests in dogs after active and passive immunization

NO.	APPROXIMATE AGE	IMMUNIZATION	SUBSEQUENT HISTORY	ANAPHYLACTIC TESTS	
				Skin	Eye
				cm.	
665	8 mo.	Vaccine	Normal for 3 mo.	—	+
675	8 mo.	Vaccine	Normal for 3 mo.	—	—
610	8 mo.	Vaccine	Normal for 3½ mo.	0.5×0.5	+
637	10 mo.	Vaccine	Normal for 3 mo.	—	—
647	10 mo.	Vaccine	Normal for 3 mo.	0.5×1.2	++
639	8 mo.	Vaccine	Normal for 3 mo.	1.0×1.0	—
657	10 mo.	Vaccine	Normal for 3 mo.	1.0×1.0	—
648	10 mo.	Vaccine	Normal for 3 mo.	—	—
597	10 mo.	Vaccine	Normal for 3½ mo.	—	—
664	9 mo.	Vaccine	Normal for 3 mo.	1.0×1.2	+
596	10 mo.	Vaccine	Normal for 4 mo.	—	±
671	8 mo.	Vaccine	Normal for 3 mo.	0.4×0.6	—
877	1½ yr.	Serum	Distemper 3 wks.	1.0×1.0	—
579	1½ yr.	Serum	Normal for 5½ mo.	2.0×2.0	—
738	6 mo.	Serum	Distemper 2 mo.	—	—
783	7 mo.	Serum	Distemper 6 wks.	—	—
704	1½ yr.	Serum	Distemper 2½ mo.	1.5×0.8	—
823	9 mo.	Serum	Distemper 5 wks.	0.6×0.3	—

Of twelve dogs receiving the vaccine and remaining free of distemper for periods of three to four months while exposed to the disease, positive skin or eye reactions or both occurred in eight (or 66.6 per cent). Unfortunately these tests were not applied to these dogs before the administration of the vaccine, but we are of the opinion that active immunization results in sensitization to bronchisepticus protein in a manner analogous to sensitization to typhoid protein among persons receiving the typhoid prophylactic.

Of the six dogs suffering with distemper and receiving anti-bronchisepticus serum, positive reactions occurred among four (or 66.6 per cent). It is probable that sensitization was due to the infection, although passive sensitization by means of the anti-serum was possible.

The skin tests were repeated about ten weeks later in a group of dogs that we were able to keep under special observation. The results are shown in table 5.

TABLE 5
Results of repeated bronchisepticin tests

NO.	APPROXIMATE AGE	FIRST SKIN TEST APRIL 6, 1916		SECOND SKIN TEST JUNE 15, 1916	
		History	Test	History	Test
			cm.		cm.
692	1 yr.	Normal 10 wks.	—	Distemper 2 wks.	0.5×0.5
718	1 yr.	Distemper 9 wks.	—	Recovered	—
681	6 mo.	Distemper 10 wks.	0.8×0.8	Recovered	—
865	9 mo.	Had distemper; normal 3 wks.	—	Normal	—
888	1 yr.	Normal 1 wk.	—	Distemper 2 mo.	1.2×1.5
783	7 mo.	Distemper: serum treatment	—	Distemper 4 mo.	1.5×1.5
738	6 mo.	Distemper: serum treatment	—	Distemper 4½ mo.	0.6×0.6
704	1½ yr.	Distemper: serum treatment	0.8×1.5	Distemper 5 mo.	1.6×1.6
596	10 mo.	Normal; vaccine	—	Normal 6 mo.	1.0×2.0
664	9 mo.	Normal; vaccine	1.0×1.2	Normal 6 mo.	1.5×2.0
597	10 mo.	Normal; vaccine	—	Normal 6 mo.	—
648	10 mo.	Normal; vaccine	—	Normal 6 mo.	—
657	10 mo.	Normal; vaccine	1.0×1.0	Normal 6 mo.	1.0×1.5
639	8 mo.	Normal; vaccine	1.0×1.0	Normal 6 mo.	0.8×0.8
647	10 mo.	Normal; vaccine	0.5×1.2	Normal 6 mo.	0.5×2.0
637	10 mo.	Normal; vaccine	—	Normal 6 mo.	—

Of these dogs two (692 and 888) had been normal at the time of the first test and reacted negatively; later both developed distemper and reacted positively in the second test. Another dog (681), which had distemper at the time of the first test, recovered and reacted negatively in the second test. Two dogs (783 and 738) suffering with distemper and receiving an anti-serum in treatment at the time of the first test and reacting negatively, later yielded positive reactions. For the most part the remaining dogs, all of which had received bronchisepticus vaccine, reacted in the second anaphylactic test in the same manner as in the first test.

SUMMARY AND CONCLUSIONS

1. A summary of the results of these anaphylactic tests with a polyvalent antigen of washed and heat-killed cultures of *B. bronchisepticus* (Ferry-McGowan) is shown in table 6.

As shown in this summary the highest percentage of positive reactions occurred among dogs suffering with distemper at the

TABLE 6

Summary of the results of the anaphylactic tests

HISTORY AT THE TIME OF THE SKIN TEST	TOTAL	RESULTS		PER CENT POSITIVE
		+	-	
Suffering with distemper.....	9	7	2	77.7
Had had distemper.....	20	12	8	60.0
Normal dogs at the time of tests.....	37	14	23	35.9
Immunized dogs.....	18	12	6	66.6

time these tests were made and among those known to have had distemper while under our direct observation.

2. Whether or not the reactions occurring among those dogs that were normal at the time of these tests, but of whose previous medical history we were ignorant and totally unable to ascertain whether or not they had distemper prior to admission to the University animal house, were true anaphylactic reactions due to sensitization with bronchisepticus protein as a result of infection, we are unable to say definitely. Owing, however, to the widespread dissemination of canine distemper and the large number of young dogs known to contract this disease, it is highly probable that some of these animals had been infected with *B. bronchisepticus* and that the reactions were an expression of cutaneous hypersensitiveness to the protein of this microörganism.

3. While we wish to record in another communication the results of our experiments bearing upon the etiological character of *B. bronchisepticus* in canine distemper either as the direct cause of this disease or as a microörganism closely associated with it in a manner analogous to the relation of streptococci to scarlet fever, we may state here that *B. bronchisepticus* is found in a large proportion of dogs suffering with distemper and that these anaphylactic tests indicate this relationship by the large proportion of infected animals displaying a condition of cutaneous hypersensitiveness to its protein.

4. While the bronchisepticin skin test may prove of practical value in the diagnosis of canine distemper and particularly as an index of a previous infection in an apparently normal ani-

mal, it is probable that it has no value as an index of immunity and that an animal presenting a positive reaction is still susceptible to relapses or recurrences of the disease (7).

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ANTIBODY PRODUCTION BY TYPHOID VACCINES

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The success attained by the use of antityphoid vaccine in military circles both in this country and abroad, naturally attracted the attention of public health officers to the use of this agent as a preventive against typhoid fever in civil life. While a very good beginning has been made, there are problems not encountered in the army that must be overcome before equally brilliant results can be hoped for in this field.

The health departments of Maryland and of Baltimore City were among the first to prepare and distribute antityphoid vaccine to the citizens of the state and city without cost. This work was begun early in 1910, and up to January 1, 1916, 22,681 complete doses of vaccine had been prepared and distributed to inhabitants of the state and city.

The greater part of this vaccine was prepared by washing off twenty-four hour old agar cultures with sterile salt solution and killing the organisms by heating at a temperature of 58°C. for one hour, or by the addition to the emulsion of 0.5 per cent phenol. The results obtained with this vaccine as a whole have been very satisfactory. Our attention, however, was directed to sensitized vaccine, which was claimed to produce a higher grade of immunity with less severe reaction, and to a vaccine described by Gay and Claypole (1) which, in addition to the above desirable features, could be administered on alternate days.

Since the results of the experimental work carried out by different authorities with sensitized and non-sensitized vaccines show considerable variation, it was deemed advisable before taking up a sensitized vaccine for general distribution to carry

out some experiments to determine, as far as possible, its efficiency. Before describing our own results we will briefly review the work of other observers with different vaccines.

Pfeiffer and Bessau (2) found that bacteria when treated with their immune serum (sensitized cultures) and inoculated into animals produced a serum poor in antibodies. Garbat and Meyer (3) inoculated rabbits and guinea-pigs with non-sensitized vaccine and produced a serum rich in agglutinin, bacteriolysin, precipitin and complement fixation body, but poor in bacteriotropin (opsonin). On the other hand the inoculation of similar doses of sensitized vaccine produced a serum containing scarcely any agglutinin, bacteriolysin, precipitin and complement fixation body, but which was strongly bacteriotropic. Broughton-Alcock (4) obtained similar results in a study of human serums. Negre (5) obtained serum with high agglutinating but low bactericidal properties by inoculating rabbits with ether killed vaccine. Animals inoculated with sensitized vaccine produced a serum with low agglutinin but high bactericidal content. Gay and Claypole (6) have made a comparative study of the protection conferred on rabbits by the inoculation of (a) whole non-sensitized vaccine; (b) sediment non-sensitized vaccine; (c) whole sensitized vaccine; (d) sediment sensitized vaccine; (e) living sensitized vaccine. After a period varying from one to three months the animals were inoculated intravenously with living typhoid cultures, and it was found that the greatest protection was produced by the sediment sensitized vaccine (d). Cecil (7) found that both sensitized living and sensitized killed typhoid bacilli were less virulent for rabbits and guinea pigs when injected intravenously than were non-sensitized killed organisms. Schottstaedt (8) in testing the antibody production of five differently prepared typhoid vaccines, two of which were sensitized and three non-sensitized, found that the three latter produced serums of much higher titer in agglutinin, bacteriolysin and opsonin, than did the sensitized vaccines. Serum containing all of these antibodies in greatest quantity was produced by a non-sensitized vaccine the organisms of which were killed by the addition

of 0.6 per cent phenol. Nichols (9) found that living sensitized vaccine was infectious for rabbits. Also, that the Rawling's strain of *B. typhosus* used in preparing the army vaccine is pathogenic, relatively avirulent and distinctly toxic. He is of the opinion that atoxic vaccine is of little value, and shares the belief of Pfeiffer and Bessau that the toxic and antigenic fractions of a vaccine are identical. Sawyer (10) reports the results of the prophylactic immunization of 8124 patients against typhoid fever in California. Of these 4967 were inoculated with sensitized and 2906 with non-sensitized vaccines, the remaining 251 being unclassified. Of the former, 4627 received the Gay-Claypole sediment sensitized vaccine. He concludes from an analysis of failure to protect against typhoid fever that the Gay-Claypole vaccine has slightly higher protective properties than does non-sensitized vaccine. He found very little difference in the reactions following the inoculation of the two vaccines.

It will be seen from the above experiments that the results obtained by different authors are quite variable, and it is impossible to arrive at any conclusion as to whether any one vaccine has a distinct advantage over the others. Unfortunately the mechanism of typhoid immunity is not well understood, and just how the injection of dead bacteria produces protection against typhoid fever is not known. That immunity persists for some time after it is impossible to demonstrate the presence of antibodies by present known methods is well established by clinical evidence. In the experimental inoculation of rabbits with typhoid vaccine Hachtel and Stoner (11) have shown that einoculation after agglutinin, bacteriolysin, and opsonin have disappeared from the blood is followed by a more rapid and prolific production of these bodies, and they conclude that the primary inoculation produces a sensitization of certain cells, so that the subsequent introduction into the body of dead or living typhoid bacilli is followed by a rapid production of antibodies by these sensitized cells in sufficient quantity to overcome, in most instances the invading organisms. Moon (12) has made a similar observation in the blood of human beings inoculated against typhoid.

Garbat (13) believes that infection is prevented by the presence of bacteriolysin assisted in some degree by the opsonin produced by the inoculation of non-sensitized vaccine. The inoculation of sensitized bacteria, according to Garbat, is followed by a breaking up and combining of the bacteria with the complement of the blood with little or no production of agglutinin, bacteriolysin or complement fixation body, and the protection is dependent upon the bacteriotropic or phagocytic activity which is stimulated by the central body or so-called endotoxin of the broken up bacteria.

Gay and Claypole (4) have obtained a specific leucocytosis in rabbits by the inoculation of sensitized typhoid bacilli, and also conclude that typhoid immunity is very largely due to phagocytosis.

Müller (15) made leucocyte counts on patients vaccinated against typhoid fever with the following results:

	AVERAGENUM- BER LEUCO- CYTES 9 CASES	AVERAGENUM- BER 4 CASES
Before inoculation.....	4260	5000
First day after inoculation.....	6170	6150
Second day after inoculation.....	4780	3920

According to these figures there is a slightly increased number of leucocytes in the first 24 hours after inoculation. It is not known whether sensitized or non-sensitized vaccine was used in inoculating these cases.

In a series of experiments carried out by Bull (16) to determine the fate of typhoid bacilli when injected intravenously into normal rabbits, it was found that the organisms disappeared from the blood stream in from one to ten minutes after inoculation. In an effort to trace the bacilli it was found that within a very short time after inoculation, from one to two minutes, the bacilli were agglutinated in the blood stream, the clumps gradually disappearing up to seven minutes, after which very few or none could be further observed. On immediately killing the animals and making smears from the organs, the clumped bacteria were found distributed in the capillaries, sinusoids, and

blood spaces of the organs, particularly in the liver, lungs and spleen, and a large proportion of the clumps had undergone phagocytosis by the polymorphonuclear leucocytes, which had accumulated in the organs following the injection of the bacteria. Free and unclumped bacilli were also found. Bull concludes that the destruction of the typhoid bacillus *intra vitam* is brought about by agglutination and intraphagocytic digestion. He does not believe there is an intracorporeal bacteriolysis, although he made plates from various tissues of the body and was unable to recover per unit of measure as great a number of bacilli as were originally found in the blood. He points out the fact that typhoid bacilli may appear and survive in the blood of human typhoid patients at a time when the blood is highly bacteriolytic for the bacilli.

In our experiments twelve rabbits were inoculated subcutaneously with three vaccines prepared as follows:

A vaccine, No. 6, was prepared from three cultures of the typhoid bacillus, Nos. 7, 29 and 30; No. 29 being a culture of the Rawling's strain used in preparing the vaccine for the army and navy, No. 7 being a Hopkins strain, and No. 30 an organism secured by blood culture from a patient with typhoid fever several years ago, both the latter possessing high binding properties. The vaccine was made by washing off twenty-four hour agar growths with normal salt solution, and the emulsion thus obtained was divided into three equal portions and labeled Nos. 6a, 6b and 6c.

No. 6a vaccine was standardized by Wright's method and the organisms killed by heating at a temperature of 58°C. for one hour. One half per cent phenol was added as a preservative.

No. 6b was sensitized by adding antityphoid immune serum to saturation, incubating for three hours and placing in the refrigerator for twenty-four hours. It was then washed in normal salt solution three times by centrifugalization, again taken up in salt solution, standardized by Wright's method, and the organisms killed by heating for one hour at 58°C. Phenol 0.5 per cent was added as a preservative.

No. 6c was sensitized and washed in the same manner as No. 6b, but the organisms were killed by the addition to the emul-

sion of an equal quantity of absolute alcohol. They were again washed in salt solution, dried *in vacuo* over sulphuric acid, ground up in a mortar, carefully weighed and again taken up in salt solution so that each cubic centimeter represented 750,000,000 organisms.

A series of four rabbits was inoculated with each of the vaccines and the blood serum was tested for agglutinin, bacteriolysin, and opsonin ten days after they had received the last inoculation. The rabbits inoculated with vaccines Nos. 6a and 6b received doses of 500,000,000, 1,000,000,000 and 1,000,000,000 each, at seven day intervals. Those inoculated with vaccine No. 6c received three doses of 1 cc. each of the 750,000,000 per cubic centimeter emulsion on alternate days.

The agglutination tests were made by the microscopic method, a twenty-four-hour old bouillon culture of No. 7 typhoid bacillus, and progressive dilutions of serum being used.

The bacteriolysins were tested by the plate method, progressive dilutions of inactivated serum, rabbit complement and a 1-10,000 dilution of a twenty-four hour old bouillon culture of No. 7 typhoid bacillus being used. The mixtures of serum, complement and bacilli were incubated for three hours, plated in neutral agar and the number of colonies counted after forty-eight hours incubation.

The opsonins were estimated by the method of Neufeld, rabbit's leucocytes being used with culture No. 7 and progressive dilutions of serum.

The results obtained with vaccine No. 6a are given in table 1, the figures in the various columns representing the highest dilution at which the various antibodies were demonstrable.

TABLE 1
Non-sensitized vaccine

RABBIT	AGGLUTININ	BACTERIOLYSIS	OPSONIN
No. 191.....	1: 5120	1: 320	1: 160
No. 192.....	1: 1280	1: 320	1: 160
No. 193.....	1: 1280	1: 40	1: 40
No. 194.....	1: 2560	1: 160	1: 80
Pooled serum of 4 rabbits.....	1: 3000	1: 200	1: 120

The results obtained with vaccine No. 6b are given in table 2.

TABLE 2
Sensitized vaccine

RABBIT	AGGLUTININ	BACTERIOLYSIN	OPSONIN
No. 195.....	1: 1280	1: 320	1: 160
No. 196.....	1: 1280	1: 160	1: 160
No. 197.....	1: 1280	1: 160	1: 80
No. 198.....	1: 1280	1: 80	1: 80
Pooled serum of 4 rabbits.....	1: 1280	1: 200	1: 120

The results obtained with vaccine No. 6c are given in table 3.

TABLE 3
Sediment sensitized vaccine, Gay

RABBIT	AGGLUTININ	BACTERIOLYSIN	OPSONIN
No. 199.....	1: 640	1: 160	1: 160
No. 200.....	1: 640	1: 160	1: 160
No. 201.....	1: 640	1: 80	1: 80
No. 202.....	1: 1280	1: 80	1: 160
Pooled serum of 4 rabbits.....	1: 800	1: 120	1: 150

On comparing the above tables it will be observed that non-sensitized vaccine, No. 6a, produced a much higher titer of agglutinin than did the sensitized vaccine No. 6b or sediment sensitized vaccine No. 6c, and that sensitized vaccine No. 6b produced higher agglutinin than did sediment sensitized vaccine No. 6c. Non-sensitized vaccine No. 6a and sensitized vaccine No. 6b produced the same titer of bacteriolysin and opsonin, the bacteriolysin being slightly higher and the opsonin slightly lower than that produced with sediment sensitized vaccine No. 6c.

In order to test further the comparative efficiency of the three vaccines, five months after having received the last dose of vaccine, all twelve rabbits were inoculated intravenously with 1 cc. of a twenty-four-hour old living culture of No. 7 typhoid bacillus. The results obtained are given in table 4.

While these experiments are too few in number to prove any-

TABLE 4

RABBIT	VACCINE USED	RESULTS
No. 191	6a Non-sensitized	Alive at the end of 8 months.
No. 192	6a Non-sensitized	Alive at the end of 8 months.
No. 193	6a Non-sensitized	Found dead 48 hours after inoculation.
No. 194	6a Non-sensitized	Alive at the end of 8 months.
No. 195	6b Sensitized	Found dead 24 hours after inoculation.
No. 196	6b Sensitized	Alive at the end of 8 months.
No. 197	6b Sensitized	Found dead 24 hours after inoculation.
No. 198	6b Sensitized	Found dead 48 hours after inoculation.
No. 199	6c Sediment sensitized	Found dead 24 hours after inoculation.
No. 200	6c Sediment sensitized	Alive at the end of 8 months.
No. 201	6c Sediment sensitized	Alive at the end of 8 months.
No. 202	6c Sediment sensitized	Found dead 24 hours after inoculation.

thing definite, they suggest that non-sensitized vaccine produces a slightly higher grade of immunity in rabbits than does sensitized, and that the degree of immunity may be approximately estimated by the production of antibodies, namely, agglutinin, bacteriolysin and opsonin.

In order to study the difference in reaction produced by the inoculation of sensitized and non-sensitized vaccines, a second vaccine was made from the same three organisms, Nos. 7, 29 and 30, and since the animal experiments showed only slight differences in the production of antibodies between the two sensitized vaccines No. 6b and 6c, this stock was divided into two equal quantities. One portion, known as No. 7a was prepared in the same manner as No. 6a, and the second portion, known as No. 7b prepared in the same manner as No. 6b.

Portions of each vaccine were furnished to the Springfield State Hospital, where very careful records are kept of all reactions following antityphoid vaccination. We were able to secure records of the temperature following the inoculation of ninety-six cases, sixty-six having been inoculated with sensitized and thirty with non-sensitized vaccines. It was impossible to secure the results following all three inoculations in every instance. The results are tabulated in table 5.

As shown in table 5, the reactions following the injection of sensitized and non-sensitized vaccines, as measured by the

TABLE 5

TEMPERATURE	SENSITIZED			NON-SENSITIZED			TOTAL SENSITIZED	TOTAL NON-SENSITIZED
	1st in.	2d in.	3d in.	1st in.	2d in.	3d in.	Per cent	Per cent
Normal.....	21	32	21	12	12	13	45.1	52.1
Normal—101° F.....	38	23	25	16	10	7	52.4	46.4
101° F—103° F.....	3	1	0	1	0	0	2.4	1.4
Over 103° F.....	0	0	0	0	0	0	0.0	0.0
Total.....	62	56	46	29	22	20		

temperature produced was a trifle less in those receiving the non-sensitized product.

A study was made of the agglutinin, bacteriolysin and opsonin present in the serums of twenty-three of these individuals. The blood was collected ten days after the third inoculation. Twelve specimens were obtained from patients inoculated with non-sensitized and eleven from those inoculated with sensitized vaccines. Of the former, six specimens were from females and six were from males, of the latter six were from females and five from males. The results obtained with non-sensitized vaccine are given in table 6.

TABLE 6

Non-sensitized vaccine

SERUM NO.	SEX	AGGLUTININ	BACTERIOLYSIN	OPSONIN
2.....	M	1: 1024	1: 128	1: 128
4.....	M	1: 1024	1: 128	1: 64
5.....	M	1: 1024	1: 128	1: 64
8.....	M	1: 1024	1: 256	1: 128
9.....	M	1: 1024	1: 128	1: 128
11.....	M	1: 2048	1: 256	1: 128
13.....	F	1: 512	1: 64	1: 32
14.....	F	1: 1024	1: 128	1: 64
15.....	F	1: 2048	1: 128	1: 128
16.....	F	1: 1024	1: 128	1: 128
19.....	F	1: 1024	1: 256	1: 128
20.....	F	1: 1024	1: 128	1: 64
Pooled serum of 12 patients.		1: 1300	1: 160	1: 100

The results obtained with sensitized vaccine are given in table 7.

TABLE 7
Sensitized vaccine

SERUM NO.	SEX	AGGLUTININ	BACTERIOLYSIN	OPSONIN
1.....	M	1: 2048	1: 256	1: 128
3.....	M	1: 512	1: 64	1: 64
6.....	M	1: 1024	1: 128	1: 128
7.....	M	1: 256	1: 64	1: 64
10.....	M	1: 256	1: 64	1: 64
12.....	F	1: 256	1: 64	1: 64
17.....	F	1: 256	1: 64	1: 32
18.....	F	1: 256	1: 128	1: 128
21.....	F	1: 1024	1: 128	1: 64
22.....	F	1: 256	1: 64	1: 64
23.....	F	1: 256	1: 128	1: 64
Pooled serum of 11 patients.		1: 600	1: 120	1: 90

Here again, as in the animal experiments the agglutinin produced by the non-sensitized vaccine is much higher than that produced by the sensitized. Also, the differences in the titer of the bacteriolysin and opsonin produced by the two vaccines is so slight as to be regarded as practically negligible.

Complement fixation tests were made with four of these specimens, two from cases that had received sensitized and two non-sensitized vaccine.

The results follow:

Serum No. 11, non-sensitized vaccine.

0.2 cc. of serum, no hemolysis.

0.1 cc. of serum, no hemolysis.

0.05 cc. of serum, no hemolysis.

Serum No. 16, non-sensitized vaccine.

0.2 cc. of serum, no hemolysis.

0.1 cc. of serum, very slight hemolysis.

0.05 cc. of serum, incomplete hemolysis.

Serum No. 17, sensitized vaccine.

0.2 cc. of serum, incomplete hemolysis.

0.1 cc. of serum, complete hemolysis.

0.0 cc. of serum, complete hemolysis.

Serum No. 21, sensitized vaccine.

0.2 cc. of serum, no hemolysis.

0.1 cc. of serum, very slight hemolysis.

0.05 cc. of serum, incomplete hemolysis.

It was intended to make complement fixation tests with all of these serums but unfortunately our work was interrupted before this could be completed.

DISCUSSION

In comparing the results obtained in these experiments with those obtained by other observers, it will be noted that in the reactions following the inoculation of non-sensitized and sensitized vaccine in human beings, our results agree with those of Sawyer, in that the difference is so slight that from a practical standpoint it is doubtful if one vaccine has any advantage over the other in this respect. The very slight difference that does exist is probably due to the idiosyncrasy of the patients inoculated, and will vary to a slight degree one way or the other with different groups of cases.

In both animal and human tests the results are in accord with those obtained by other writers in that non-sensitized vaccine produced a higher titer of agglutinin than did sensitized.

In regard to the bacteriolysin and opsonin produced by non-sensitized and sensitized vaccines, in our experiments both human and animal, the differences between the two were so slight that they were probably due to the methods employed in making the tests or to unavoidable errors in technique, and it cannot be said that either vaccine possessed any marked advantage over the other in the production of these two antibodies. These results do not agree with those obtained by Garbat and Meyer, Broughton Alcock, and Gay and Claypole, who found that sensitized vaccine produced serums rich in opsonin but poor in bacteriolysin, and non-sensitized produced serums rich in bacteriolysin but comparatively poor in opsonin. Nor do they concur with those of Schottstaedt, who found that non-sensitized vaccine produced serums richer in both bacteriolysin and opsonin than did sensitized.

From these various results it must be assumed that while sensitized vaccine does sometimes produce serums richer in opsonin and poorer in bacteriolysis than does non-sensitized, this is not constant and the reverse may occur. It seems pos-

sible that so far as the production of bacteriolysin and opsonin is concerned, it may vary under different conditions and with different animals.

Assuming this to be a fact, since the superiority of sensitized vaccine is supposed to be due almost entirely to its opsonic producing qualities, and since our experiments as well as others show that this quality is not constant, the question as to whether it possess advantages over non-sensitized vaccine in the prophylactic immunization against typhoid fever is doubtful.

It is well known that so far as the production of immune bodies is concerned, the same phenomena follow the parenteral introduction of killed typhoid bacilli into the body and the entrance of the living infectious organism through the digestive tract. The experiments of Bull show that agglutinin plays a more important part in the production of immunity than has heretofore been credited to it. It has long been believed that the disappearance of typhoid bacilli from the blood in typhoid fever was due to the activity of the bacteriolysins. These have been demonstrated *in vitro* in the serums of typhoid patients by Stern and Korte (17), Hahn (18), Korte and Steinberg (19), Dennison (20), and others. In four cases studied by Dennison bacteriolysin was demonstrated at the height of the disease at dilutions of 1-1,000,000, 1-500,000, 1-50,000, and 1-50,000 respectively. Therefore, it seems probable that in the prophylactic inoculation against typhoid fever bacteriolysin may also play an important rôle.

After a study of the results obtained by other observers as well as by ourselves, we cannot accept the theory that typhoid immunity is due to phagocytic activity alone, but believe that the mechanism is a multiple one in which agglutinin, bacteriolysin, opsonin and perhaps still other immune bodies all take an active part.

CONCLUSIONS

As measured by the production of agglutinin, bacteriolysin, and opsonin, sensitized vaccine possesses no distinct advantage over non-sensitized vaccine in the production of prophylactic typhoid immunity.

The reactions following the injections of prophylactic typhoid vaccine are about the same with both the sensitized and non-sensitized product.

Owing to lack of concrete evidence that prophylactic typhoid immunity is due wholly to phagocytic activity, and that sensitized vaccine produces an increased phagocytosis, we shall still continue to recommend the use of non-sensitized vaccine.

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CAN A SPECIFIC NEUROTOXIN BE OBTAINED FROM THE CORPUS STRIATUM?

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In a study of the thermogenic functions of the corpus striatum I have been led into a reinvestigation of the question of the occurrence of specific neurotoxins. Certain substances bring about a marked change of body temperature when applied to the corpus striatum and do not produce this effect when applied to other parts of the brain (1). This would indicate a difference in chemical composition between the corpus striatum and the other parts. It is conceivable that the characteristic differences should depend upon the protein constituents and hence it might be possible, by suitable methods, to induce the formation of specific neurotoxins in case such substances really occur.

Delezenne (2) described a neurotoxic serum obtained from a rabbit by repeated intraperitoneal or subcutaneous injection of an emulsion of dog brain. This serum, when injected into the marginal vein of a dog's ear, proved fatal in 5 to 10 minutes in doses of 0.2 cc. per kilo of body weight. Subcutaneous injection of 0.3 to 0.4 cc. caused death in one hour. Intracerebral injection of 0.005 cc. per kilo killed the dog in 5 to 10 minutes; 0.0001 cc. in a few days. Delezenne states that the serum is fifty times more toxic when injected directly into the brain. His detailed studies of the nervous symptoms and the lesions produced by injection of neurotoxic serum were made on the dog with doses of 0.5 to 0.6 cc. of neurotoxic rabbit serum injected into the brain anterior to the cerebellum. After a few minutes, convulsions and partial paralysis of the limbs took place. There was rapid suppression of all activities of the central nervous system, especially the functions of the medulla.

Death in most cases followed in 24 hours. In a number of cases the animals recovered in 3 to 4 days.

Enriquez and Sicard (3) attempted to obtain a rabbit serum that would be neurotoxic for the dog. They were successful in getting only a weakly active serum. Revanna (4) had similar difficulties. Centanni (5), however, reported having obtained a neurotoxic serum by injecting a sheep intraperitoneally with an emulsion of rabbit brain.

Armand-Delille (6) carried out an extended set of experiments injecting an emulsion of the brain of the dog into animals of several different species, using the exact method of Delezenne. He found that with certain animals it was impossible to obtain any neurotoxic serum. Experiments on sheep, rabbits, dogs and guinea pigs were successful. His most complete series of experiments was with dogs and guinea pigs. An emulsion of 1 gram in 6 cc. physiological salt solution of cerebrum, cerebellum, and medulla of a dog was injected intraperitoneally about every four days into guinea pigs and the injection was repeated 4 to 6 times. Six to seven days after the last injection these animals were bled. Of the serum obtained, 0.3 to 1.2 cc. per kilo was injected intracerebrally into a dog. After injection the dog remained normal about 15 minutes. Then torpor came on lasting 40 minutes to 3 hours. This was followed by 5 minutes of intense convulsions. A series of convulsions followed by intervals of coma, each more intense than the last, preceded death, which came in 1 to 24 days. In a few cases the animal recovered; in others there were convulsions and no coma and vice versa. His histological study of the brain of a dog that died several days after injection showed vascular congestion, infiltration and extreme chromatolysis and alteration of the nerve fibers. A control experiment with injections of normal guinea pig serum produced no symptoms even when increased to 2 to 3 cc. per kilo of body weight. Goldbaum (7) confirmed the results of Delezenne and Armand-Delille.

The above observations seemed to show that a serum prepared by repeated intraperitoneal injections of the brain substance of an animal of another species when injected intracerebrally into

an animal of the species from which the brain substance was taken caused extreme nervous disorders, generally followed by death.

My experiments were based on the general method of Delezenne and were at first performed in the following way: An adult cat was bled by cutting the carotids and jugulars. The corpus striatum was taken out antiseptically and washed in physiological salt solution to free it from blood. It was then ground in a mortar until a homogeneous mass of about five grams was obtained. To this was added five times its volume (about 25 cc.) of physiological salt solution, aseptic precautions being taken with all instruments and vessels used. This emulsion was injected intraperitoneally or subcutaneously, the results being the same in either case, into four of five rabbits. Each animal received 5 cc. (about 1 gram of nerve material). The injections were repeated every day for from 4 to 5 days, varying in different cases.

After 10 to 14 days, or in several cases a longer period, the rabbits were bled from the carotid into sterile test tubes. The tubes were left 18 to 24 hours at 10°C. and then the serum was drawn into sterile pipettes in which it could be kept. About 15 cc. of serum was, with antiseptic precautions, injected subcutaneously into a cat whose normal range of temperature had been previously determined. The temperature was taken every twenty minutes for from 6 to 12 hours after injection.

Four series of experiments, with five rabbits and three cats in each, were made, the results being practically the same in every case. In one case, which may be taken as typical, injection of 5 cc. of immunized serum gave a rise of 0.4 to 0.5°C. Injection of the same amount of normal rabbit serum gave a rise of 0.3–0.4°C. This difference is not at all of the order of magnitude to be expected if a specific neurotoxin had really been obtained; for injury to the corpora striata, as found by White (8), Ott (9), Nikolaïdes (10), and others, brings about a rise of temperature of 2 to 7°C. The small rise of temperature in my results cannot be interpreted as an indication, therefore, that cytolysis had taken place in the corpus striatum or that a specific

neurotoxin to the corpus striatum could be obtained. Neither nervous symptoms nor death resulted in any case.

These experiments were repeated a sufficient number of times to make very definite the negative outcome. Positive results might be called in question as the result of faulty technic, but negative results under the conditions of the experiment can mean only that the neurotoxins do not exist.

In order to determine, if possible, whether the extreme nervous symptoms obtained by Delezenne, Armand-Delille and others were due to some cause other than that of a neurotoxin, the following experiments were performed:

1. 1 cc. of serum prepared as in the first series was injected into the cerebrum of a cat just anterior to the cerebellum and to the right of the median line. No nervous symptoms nor other effects of the injection were noted.

2. In another set of experiments the exact method of Delezenne was used. Rabbits were injected every four days for four times with the cerebellum and medulla of a cat's brain. After 12 days one rabbit was bled. Three cubic centimeters of the serum obtained was injected intracerebrally into a cat. No nervous symptoms resulted.

Another rabbit of the same series, which from some unknown cause became extremely sick, was bled. Its serum was injected intracerebrally into another cat. The cat died after the second day. Examination of the brain showed marked infiltration.

3. (a) Normal cat corpuscles were treated with the rabbit serum prepared in experiments 1 and 2. The result was negative as to hemolysis and positive as to agglutination.

- (b) Normal cat serum treated with normal rabbit serum showed neither hemolysis nor agglutination.

- (c) Normal cat serum treated with prepared rabbit serum showed marked precipitation.

- (d) Normal cat serum treated with normal rabbit serum showed only very slight precipitation.

4. Hemolytic rabbit serum was prepared by repeated injection of 2 cc. of cat blood. 0.2 cc. of this hemolytic serum was injected intracerebrally into a cat. The cat never recovered

from the anaesthetic. It appeared drowsy and was unable to stand erect. The torpor increased on the following day. It died on the third day.

The above experiments show that a strongly hemolytic serum caused torpor and finally death when injected intracerebrally. On the other hand, a non-hemolytic serum prepared by injection of nerve tissue had no noticeable effect. The results obtained by the workers cited above might, therefore, be due to a hemolytic or other toxic action of the serum and not to neurotoxins. The infiltration, vascular congestion and chromatolysis could all be produced by hemolysis. The so-called exceptional cases reported by Delezenne and Armand-Delille in which the animals recovered in 2 to 4 days might well be taken as positive results indicating that neurotoxins had not been obtained. The other cases cannot be cited as proof of the presence of neurotoxins.

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A CONTRIBUTION TO THE ACTION OF AMIDOACIDS, PEPTIDS AND PROTEINS ON HEMOLYSIS BY COBRAVENOM

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INTRODUCTION

We (1) have shown that the several products of the disintegration of the proteins have, in certain quantities, a thromboplastic action. Now many authors (Noff (2), DeWaele (3), etc.), claim some very close connections between bloodclotting, hemolysis, anaphylaxis and the numerous immunological reactions. It has, therefore, appeared interesting to us to inquire whether the amidoacids, the peptids, and the proteoses exert an action on hemolysis.

In this communication (4), we shall describe only the experiments that we have made with cobra venom.

TECHNIQUE

We used decimolecular solutions of glycocoll, alanin, leucin and phenylglycocoll; twentieth molecular solutions of diglycin, triglycin and leucylglycin; saturated solutions of glycyltryptophan, tetraglycin, and pentaglycin, and 1 per cent solution of protoalbumose and heteroalbumose. These proteoses were prepared after the method of Pick (5).

All of these solutions were made with physiological salt solution (0.85 per cent) and accurately neutralized towards litmus.

In each experiment, we set up a series of test tubes. Each tube contained 0.1 cc. of a 0.4 per cent solution of cobra venom, and 1 cc. of one or the other of the preceding solutions of the split products of proteins, either as it was or in a variable degree of dilution with physiological saline solution. In every test tube

was placed one drop of a suspension of the red corpuscles of guinea-pig, man, dog, rabbit, sheep, calf or ox.

In some experiments, we added to a part of the test tubes, before the addition of the suspension of red corpuscles, 0.1 cc. of the serum of guinea-pig or of rabbit, either as it was or after inactivation by heating at 56°C.

In each experiment, a tube containing 1 cc. of cobra venom and a drop of the employed suspension of red corpuscles was prepared. We prepared also a set of control tubes, without cobra venom, containing the different experimental solutions of amidoacids, peptids or proteoses and red corpuscles alone or with guinea-pig's serum or rabbit's serum either as it was or after inactivation.

All the test tubes were closed with little India rubber stoppers. The tubes remained at the temperature of the laboratory (18° to 20°C.).

We noted approximately the beginning of the hemolysis only in the first experiments. This unfortunately is not easy with a great number of test tubes. Therefore we give all our attention to the determination of the intensity of the hemolysis; first, after 1½ to 5 hours, and then after 18 to 20 hours. This was done by a method similar to the one that Madsen proposed for testing the hemolytic properties of tetanolysin. In each experiment, we diluted the suspension of red corpuscles with the quantity of water necessary to complete hemolysis. This red fluid was taken as the degree 10 of intensity of hemolysis. On the other hand, the suspension of red corpuscles without addition of water and also without hemolysis corresponded to the degree 0. Through addition of 1 to 9 parts of water to the suspension of red corpuscles, we obtained several fluids with degrees of hemolysis corresponding with 1 to 9 (or approximately from 10 to 90 per cent of complete hemolysis). By comparing the different test tubes with this color scale, we could determine approximately the intensity of hemolysis in each test tube.

As is well known, the resistance of the red corpuscles is not the same in the different animal species. We shall present our experiments in the order of increasing resistance towards the hemolytic action of cobra venom, namely, first the experiments

with the red corpuscles of the guinea-pig, then successively the experiments with those of man, dog, rabbit, sheep, calf and ox.

We never observed hemolysis in the control tubes without cobravenom, so that it is unnecessary to speak further of these.

Experiments with red corpuscles of the guinea-pig

We have given, in the form of tables, the three experiments that were made with these corpuscles.

In Experiment 1, the beginning of the hemolysis was accelerated by the addition of glycyltryptophan, of protoalbumose, and above all of leucin. This acceleration was most marked with the large dose of leucin and with the small doses of protoalbumose and of glycyltryptophan. The addition of 0.5 to 1.0 cc. of diglycin or of 0.1 to 1.0 cc. of triglycin or leucylglycin, on the contrary, occasioned a delay in the beginning of the hemolysis.

The intensity of the hemolysis is notably increased after addition of glycyltryptophan and above all of leucin. After the addition of leucylglycin or of triglycin, one observes after 2 hours no hemolysis by cobravenom and the intensity of the hemolysis is not very great after 19 hours. The large doses of these peptids exert a greater inhibitory action than the others. We observe also no hemolysis after 2 hours in the test tubes containing 0.5 to 1.0 cc. of diglycin and 0.9 to 0.1 cc. of alanin. After the same time, the intensity of the hemolysis is less in the tubes containing also 1 cc. of glycocoll, 0.1 cc. of diglycin, 0.1 to 0.5 cc. of alanin or 0.5 to 1 cc. of protoalbumose than in the control tube with physiological saline and cobravenom. After 19 hours, the decrease in the intensity of the hemolysis exists in the tubes containing 1 cc. of glycocoll, 0.1 to 1 cc. of diglycin (the large dose being the most active), 0.5 to 1 cc. of alanin or 0.5 to 1 cc. of protoalbumose.

The Experiment 2 shows that the hemolysis of the red corpuscles of guinea-pig did not begin after 3 hours without addition of serum, but was complete after 19 hours. The beginning of the hemolysis was accelerated by 0.1 to 1 cc. of glycyltryptophan (the most by the small dose), 0.1 to 1 cc. of leucin (the most by the large dose), 0.1 cc. of alanin, 0.5 to 1 cc. of heteroalbumose

Experiment 1

EVERY TEST TUBE CONTAINS 0.1 CC. OF 4 PER CENT SOLUTION OF COBRAVENOM AND 1 DROP OF SUSPENSION OF RED CORPUSCLES OF GUINEA-PIG AND FURTHER	DEGREE OF HEMOLYSIS AFTER		
	Beginning after about	2 hours	10 hours
1 cc. salt*	2 hours (short)	4	6
0.9 cc. salt + 0.1 cc. glycocoll.....	2 hours	4	6
0.5 cc. salt + 0.5 cc. glycocoll.....	1½ hours	5	6
0.1 cc. salt + 0.9 cc. glycocoll.....	2 hours	4	6
1 cc. glycocoll.....	2 hours	Between 2 and 3	4
0.9 cc. salt + 0.1 cc. diglycin.....	2 hours	Between 2 and 3	Between 5 and 6
0.5 cc. salt + 0.5 diglycin.....	Between 2 and 19 hours	0	4
0.1 cc. salt + 0.9 cc. diglycin.....	Between 2 and 19 hours	0	2
1 cc. diglycin.....	Between 2 and 19 hours	0	2
0.9 cc. salt + 0.1 cc. triglycin.....	Between 2 and 19 hours	0	4
0.5 cc. salt + 0.5 cc. triglycin.....	Between 2 and 19 hours	0	2
0.1 cc. salt + 0.9 cc. triglycin.....	Between 2 and 19 hours	0	2
1 cc. triglycin.....	Between 2 and 19 hours	0	2
0.9 cc. salt + 0.1 cc. glycyltryptophan.....	1 hour	5	8
0.5 cc. salt + 0.5 cc. glycyltryptophan.....	1½ hours	5	8
0.1 cc. salt + 0.9 cc. glycyltryptophan.....	1½ hours	5	Between 8 and 9
1 cc. glycyltryptophan.....	1½ hours	Between 4 and 5	Between 8 and 9
0.9 cc. salt + 0.1 cc. leucin.....	1 hour	7	Between 8 and 9
0.5 cc. salt + 0.5 cc. leucin.....	¾ hour	9	10
0.1 cc. salt + 0.9 cc. leucin.....	¾ hour	9	10
1 cc. leucin.....	25 minutes	9	10
0.9 cc. salt + 0.1 cc. leucylglycin.....	Between 2 and 19 hours	0	2
0.5 cc. salt + 0.5 cc. leucylglycin.....	Between 2 and 19 hours	0	2
0.1 cc. salt + 0.9 cc. leucylglycin.....	Between 2 and 19 hours	0	2
1 cc. leucylglycin.....	Between 2 and 19 hours	0	Between 1 and 2
0.9 cc. salt + 0.1 cc. alanin.....	2 hours	3	6
0.5 cc. salt + 0.5 cc. alanin.....	2 hours	3	6
0.1 cc. salt + 0.9 cc. alanin.....	Between 2 and 19 hours	0	4
1 cc. alanin.....	Between 2 and 19 hours	0	4
0.9 cc. salt + 0.1 cc. protoalbumose.....	1 hour	4	6
0.5 cc. salt + 0.5 cc. protoalbumose.....	1½ hours	3	6
0.1 cc. salt + 0.9 cc. protoalbumose.....	1½ hours	3	4
1 cc. protoalbumose.....	1½ hours	Between 2 and 3	Between 2 and 3

* Salt=physiological saline solution.

Experiment 2

EACH TEST TUBE CONTAINS 0.1 CC. OF 4 PER CENT SOLUTION OF COBRAVENOM AND 1 DROP OF SUSPENSION OF RED CORPUSCLES OF GUINEA PIG AND FURTHER	WITH NO SERUM OF GUINEA PIG		WITH 0.1 CC. OF SERUM OF GUINEA-PIG	
	Intensity of hemolysis after			
	3 hours	19 hours	3 hours	19 hours
1 cc. salt.....	0	10	Between 4 and 5	10
0.9 cc. salt + 0.1 cc. glycocoll.....	0	10	5	10
0.5 cc. salt + 0.5 cc. glycocoll.....	0	10	Between 6 and 7	10
1 cc. glycocoll.....	0	7	7	10
0.9 cc. salt + 0.1 cc. diglycin.....	0	6	4	10
0.5 cc. salt + 0.5 cc. diglycin.....	0	Between 1 and 2	4	10
1 cc. diglycin.....	0	Between 1 and 2	3	10
0.9 cc. salt + 0.1 cc. triglycin.....	0	Between 3 and 4	5	10
0.5 cc. salt + 0.5 cc. triglycin.....	0	Between 0 and 1	2	10
1 cc. triglycin.....	0	0	2	10
0.9 cc. salt + 0.1 cc. glycytryptophan.....	Between 4 and 5	10	Between 5 and 6	10
0.5 cc. salt + 0.5 cc. glycytryptophan.....	Between 2 and 3	10	6	10
1 cc. glycytryptophan.....	3	7	5	10
0.9 cc. salt + 0.1 cc. leucin.....	Between 2 and 3	10	Between 3 and 4	6
0.5 cc. salt + 0.5 cc. leucin.....	4	10	5	8
1 cc. leucin.....	5	10	7	10
0.9 cc. salt + 0.1 cc. leucylglycin.....	0	8	5	10
0.5 cc. salt + 0.5 cc. leucylglycin.....	0	6	5	10
1 cc. leucylglycin.....	0	0	4	8
0.9 cc. salt + 0.1 cc. alanin.....	Between 1 and 2	9	5	9
0.5 cc. salt + 0.5 cc. alanin.....	0	7	Between 5 and 6	10
1 cc. alanin.....	0	5	Between 4 and 5	10
0.9 cc. salt + 0.1 cc. heteroalbumose.....	0	0	0	0
0.5 cc. salt + 0.5 cc. heteroalbumose.....	3	10	0	0
1 cc. heteroalbumose.....	5	10	Between 0 and 1	10
0.9 cc. salt + 0.1 cc. protoalbumose.....	1	10	Between 5 and 6	10
0.5 cc. salt + 0.5 cc. protoalbumose.....	5	10	Between 5 and 6	10
1 cc. protoalbumose.....	Between 0 and 1	10	Between 5 and 6	10

(the most by the large dose), 0.1 to 1 cc. of protoalbum (the most by the small dose).

After 19 hours, we observe no hemolysis in the tubes containing 1 cc. of triglycin, 1 cc. of leucylglycin or 0.1 cc. of heteroalbumose. As the hemolysis is already complete (10) after 19 hours in the control tube, it is natural that we do not observe any action by the addition of 0.1 to 0.5 cc. of glycyltryptophan, of 0.1 to 1 cc. of leucin, of 0.5 to 1 cc. of heteroalbumose or of 0.1 to 1 cc. of protoalbumose, because at these doses all these products have already accelerated the hemolysis after 3 hours.

The intensity of the hemolysis is decreased by 1 cc. of glycoll, more by 0.1 to 1 cc. of diglycin (the most by the large dose) and still more by 0.1 to 0.5 cc. of triglycin (the most by the large dose). The inhibitory action is here also in strong connection with the complexity of the added product, diglycin being more active than glycin, and triglycin than diglycin. This was already shown in Experiment 1, but it is more apparent in Experiment 2. In both experiments leucylglycin and alanin exert an inhibitory action on the hemolysis, chiefly in the large doses. In Experiment 2, 1 cc. of glycyltryptophan exerts a decreasing action on the intensity of the hemolysis after 19 hours, contrary to the results obtained after 3 hours in this experiment and 2 and 19 hours in Experiment 1.

The addition of serum accelerates notably the hemolysis after 3 hours in the control tube containing physiological saline serum and cobra venom and in all the other tubes with the exception of these containing 0.1 to 0.5 cc. of heteroalbumose. The action of the serum on the hemolysis by cobra venom is increased by glycoll (the most in the large dose) and slightly by 0.1 cc. of triglycin, 0.1 to 0.5 cc. of glycyltryptophan (the most in the small dose), 0.5 to 1 cc. of leucin (the most in the large dose), 0.1 cc. of leucylglycin, 0.1 to 0.5 cc. of alanin, 0.1 to 0.5 cc. of protoalbumose. On the contrary this action is decreased by 0.1 to 1 cc. of diglycin (the most in the large dose), 0.5 to 1 cc. of triglycin, 0.1 cc. of leucin, 1 cc. of leucylglycin. After 19 hours, we observe everywhere complete hemolysis with the exception of the tubes containing 0.1 cc. of alanin, 1 cc. of leucylglycin, 0.1 to 0.5

Experiment 3

EACH TEST TUBE CONTAINS 0.1 CC. OF 4 PER CENT SOLUTION OF COBRAVENOM AND 1 DROP OF SUSPENSION OF RED CORPUSCLES OF GUINEA PIG AND FURTHER	AND NO SERUM OF GUINEA PIG		AND 0.1 CC. OF SERUM OF GUINEA PIG		AND 0.1 CC. OF PREVIOUSLY INACTIVATED SERUM OF GUINEA PIG	
	Intensity of hemolysis after		Intensity of hemolysis after		Intensity of hemolysis after	
	2 hours	18 hours	2 hours	18 hours	2 hours	18 hours
1 cc. salt.....	0	2	10	10	1	5
0.9 cc. salt + 0.1 cc. glycocoll.....	1	3	10	10	1	5
0.5 cc. salt + 0.5 cc. glycocoll.....	0	2	10	10	1	5
1 cc. glycocoll.....	0	2	10	10	1	5
0.9 cc. salt + 0.1 cc. diglycin.....	0	2	10	10	1	5
0.5 cc. salt + 0.5 cc. diglycin.....	0	1	9	10	1	5
1 cc. diglycin.....	0	Between 0 and 1	8	10	Between 0 and 1	5
0.9 cc. salt + 0.1 cc. phenylglycocoll.....	6	Between 9 and 10	10	10	7	10
0.5 cc. salt + 0.5 cc. phenylglycocoll.....	10	10	10	10	10	10
1 cc. phenylglycocoll.....	10	10	10	10	10	10
0.9 cc. salt + 0.1 cc. glycyltryptophan.....	4	10	10	10	Between 2 and 3	6
0.5 cc. salt + 0.5 cc. glycyltryptophan.....	4	10	10	10	3	10
1 cc. glycyltryptophan.....	3	10	10	10	5	10
0.9 cc. salt + 0.1 cc. leucin.....	4	10	10	10	3	8
0.5 cc. salt + 0.5 cc. leucin.....	5	10	10	10	4	10
1 cc. leucin.....	7	10	10	10	6	10
0.9 cc. salt + 0.1 cc. heteroalbumose.....	1	4	8	10	1	5
0.5 cc. salt + 0.5 cc. heteroalbumose.....	0	3	6	8	1	5
1 cc. heteroalbumose.....	0	3	3	6	1	5
0.9 cc. salt + 0.1 cc. protoalbumose.....	3	4	10	10	Between 1 and 2	5
0.5 cc. salt + 0.5 cc. protoalbumose.....	4	10	10	10	Between 1 and 2	5
1 cc. protoalbumose.....	6	10	10	10	2	6

cc. of leucin, where the intensity of the hemolysis is decreased, and of the tubes containing 0.1 to 0.5 cc. of heteroalbumose where no hemolysis at all exists.

The Experiment 3 shows but a very late and very slight hemolysis in the control tube without serum. The addition of guinea pig's serum is enough to produce complete hemolysis in 2 hours time. Heating of the serum at 56 degrees diminishes this action in a very great proportion, so that the intensity of the hemolysis is but 1 after 2 hours and 5 after 18 hours.

Without any addition of serum, the addition of 0.1 cc. of glycocoll accelerates and increases slightly the intensity of the hemolysis. The addition of 0.5 to 1 cc. of glycocoll has no effect at all. This is also the case with the addition of 0.1 cc. of diglycin. The addition of 0.5 cc. of diglycin and, in greater degree, the addition of 1 cc. of this peptid decreases the intensity of the hemolysis. All the other products of the disintegration of the proteins increase the intensity of the hemolysis and accelerate the beginning of the hemolysis. This action is most marked after the addition of phenylglycocoll; then come in decreasing order leucin, protoalbumose (the most with the greatest dose), glycyltryptophan and in a slight degree heteroalbumose (the most with the smallest dose.)

After the addition of serum, one can see the inhibitory effects of the addition of 0.5 to 1 cc. of diglycin or of 0.1 cc. of heteroalbumose only on the beginning of the hemolysis whereas a similar effect is seen to be produced on the intensity of the hemolysis after 18 hours by 0.5 to 1 cc. of heteroalbumose.

When the serum is heated at 56 degrees, glycocoll and heteroalbumose exert no effect at all. This is also the case with the addition of 0.1 cc. of diglycin. The addition of 0.5 to 1 cc. of diglycin produces some delay in the beginning of the hemolysis. Protoalbumose in small and medium doses accelerates a little the beginning of the hemolysis and in the large dose increases also the intensity of the hemolysis. Phenylglycocoll, with the addition of serum, has the same strong accelerating action on the beginning and increasing effect on the intensity of the hemolysis as without any addition of serum. One observes almost the

same effects upon the addition of glycytryptophan or of leucin in the test tubes without serum or with serum previously heated at 56 degrees.

Dans l'ensemble, l'hémolyse des hématies de cobaye est notablement accélérée et accrue en intensité par le phénylglycocolle, la leucine, le glycytryptophane et la protoalbumose. L'hémolyse s'effectue plus vite en présence de fortes que de faibles quantités de phénylglycocolle et de leucine. L'hétéroalbumose tend à accroître légèrement l'intensité de l'hémolyse, l'alanine au contraire à la diminuer quelque peu. Le glycocolle et surtout la diglycine, la triglycine et la leucylglycine entravent l'hémolyse, et cela d'autant plus que la concentration est plus forte.

En présence de 0.1 cc. de sérum frais, l'action accélératrice de la protoalbumose et du glycytryptophane et l'action inhibitrice des fortes concentrations de diglycine, de triglycine et de leucylglycine ne se font plus guère sentir que dans de faibles limites. L'hétéroalbumose ne montre plus que des effets nocifs dans ces conditions.

La présence de sérum inactive n'empêche pas l'accélération de l'hémolyse par le phénylglycocolle. Elle diminue par contre les effets favorables du glycytryptophane et de la leucine et annihile presque ceux de la protoalbumose. Les effets nocifs de la diglycine sont beaucoup atténués. Le glycocolle et l'hétéroalbumose ne paraissent plus exercer aucune action.

Experiments with red corpuscles of man

Nous rapportons trois expériences de ce genre. Les hématies proviennent de sang recueilli aseptiquement chez trois personnes en parfaite santé.

L'hémolyse s'est, comme chez le cobaye, déjà produite en l'absence de sérum. L'addition de sérum frais de cobaye a accéléré la vitesse de l'hémolyse. L'addition de sérum chauffé au préalable à 56 degrés a retardé la rapidité de ce phénomène et en a diminué l'intensité.

Le phénylglycocolle a notablement accéléré l'hémolyse (et

Experiment 4

EACH TEST TUBE CONTAINS 0.1 CC. OF 4 PER CENT SOLUTION OF COBRAVENOM AND 1 DROP OF SUSPENSION OF RED CORPUSCLES OF MAN, AND FURTHER	INTENSITY OF HEMOLYSIS AFTER	
	2 hours	18 hours
1 cc. salt.....	0	4
0.9 cc. salt + 0.1 cc. glycocoll.....	1	5
0.5 cc. salt + 0.5 cc. glycocoll.....	1	5
1 cc. glycocoll.....	1	5
0.9 cc. salt + 0.1 cc. diglycin.....	0	Between 3 and 4
0.5 cc. salt + 0.5 cc. diglycin.....	0	Between 2 and 3
1 cc. diglycin.....	0	2
0.9 cc. salt + 0.1 cc. phenylglycocoll.....	Between 0 and 1	Between 5 and 6
0.5 cc. salt + 0.5 cc. phenylglycocoll.....	5	10
1 cc. phenylglycocoll.....	7	10
0.9 cc. salt + 0.1 cc. glycytryptophan...	Between 0 and 1	3
0.5 cc. salt + 0.5 cc. glycytryptophan...	0	Between 2 and 3
1 cc. glycytryptophan.....	0	2
0.9 cc. salt + 0.1 cc. leucin.....	2	Between 6 and 7
0.5 cc. salt + 0.5 cc. leucin.....	4	10
1 cc. leucin.....	6	10
0.9 cc. salt + 0.1 cc. alanin.....	Between 0 and 1	4
0.5 cc. salt + 0.5 cc. alanin.....	Between 0 and 1	4
1 cc. alanin.....	1	4
0.9 cc. salt + 0.1 cc. heteroalbumose.....	1	4
0.5 cc. salt + 0.5 cc. heteroalbumose.....	Between 0 and 1	3
1 cc. heteroalbumose.....	0	2
0.9 cc. salt + 0.1 cc. protoalbumose.....	Between 0 and 1	4
0.5 cc. salt + 0.5 cc. protoalbumose.....	6	Between 9 and 10
1 cc. protoalbumose.....	8	10

Experiment 5

EACH TEST TUBE CONTAINS 0.1 CC. OF 4 PER CENT SOLUTION OF COBRAVENOM AND 1 DROP OF SUSPENSION OF RED CORPUSCLES OF MAN, AND FURTHER	INTENSITY OF HEMOLYSIS AFTER	
	2½ hours	18 hours
1 cc. salt.....	Between 0 and 1	10
0.9 cc. salt + 0.1 cc. glycocoll.....	1	10
0.5 cc. salt + 0.5 cc. glycocoll.....	Between 0 and 1	10
1 cc. glycocoll.....	Between 0 and 1	10
0.9 cc. salt + 0.1 cc. diglycin.....	0	8
0.5 cc. salt + 0.5 cc. diglycin.....	0	7
1 cc. diglycin.....	0	5
0.9 cc. salt + 0.1 cc. triglycin.....	0	10
0.5 cc. salt + 0.5 cc. triglycin.....	0	Between 9 and 10
1 cc. triglycin.....	0	Between 8 and 10
0.9 cc. salt + 0.1 cc. tetraglycin.....	0	3
0.5 cc. salt + 0.5 cc. tetraglycin.....	0	0
1 cc. tetraglycin.....	0	0
0.9 cc. salt + 0.1 cc. pentaglycin.....	0	9
0.5 cc. salt + 0.5 cc. pentaglycin.....	0	6
1 cc. pentaglycin.....	0	4
0.9 cc. salt + 0.1 cc. phenylglycocoll.....	Between 1 and 2	10
0.5 cc. salt + 0.5 cc. phenylglycocoll.....	Between 6 and 7	10
1 cc. phenylglycocoll.....	Between 8 and 9	10
0.9 cc. salt + 0.1 cc. glycytryptophan...	4	10
0.5 cc. salt + 0.5 cc. glycytryptophan...	Between 0 and 1	10
1 cc. glycytryptophan.....	Between 0 and 1	10
0.9 cc. salt + 0.1 cc. leucin.....	Between 3 and 4	10
0.5 cc. salt + 0.5 cc. leucin.....	6	10
1 cc. leucin.....	Between 6 and 7	10
0.9 cc. salt + 0.1 cc. alanin.....	Between 0 and 1	10
0.5 cc. salt + 0.5 cc. alanin.....	Between 0 and 1	10
1 cc. alanin.....	Between 1 and 2	10
0.9 cc. salt + 0.1 cc. heteroalbumose.....	Between 2 and 3	10
0.5 cc. salt + 0.5 cc. heteroalbumose.....	0	Between 9 and 10
1 cc. heteroalbumose.....	0	8
0.9 cc. salt + 0.1 cc. protoalbumose.....	2	10
0.5 cc. salt + 0.5 cc. protoalbumose.....	3	10
1 cc. protoalbumose.....	4	10

Experiment 6

EACH TEST TUBE CONTAINS 0.1 CC. OF 4 PER CENT SOLUTION OF COBRAVENOM AND 1 DROP OF SUSPENSION OF RED CORPUSCLES OF MAN AND FURTHER	AND NO SERUM OF GUINEA PIG		AND 0.1 CC. OF SERUM OF GUINEA PIG		AND 0.1 CC. OF PREVIOUSLY INACTIVATED GUINEA PIG'S SERUM	
	Intensity of hemolysis after		Intensity of hemolysis after		Intensity of hemolysis after	
	3 hours	18 hours	3 hours	18 hours	3 hours	18 hours
1 cc. salt.....	Bet. 1 and 2	10	7	10	Bet. 1 and 2	7
0.9 cc. salt + 0.1 cc. glycocoll.....	4	10	7	10	Bet. 1 and 2	7
0.5 cc. salt + 0.5 cc. glycocoll.....	3	10	7	10	Bet. 1 and 2	7
1 cc. glycocoll.....	Bet. 2 and 3	10	7	10	Bet. 1 and 2	7
0.9 cc. salt + 0.1 cc. diglycin.....	Bet. 1 and 2	10	7	10	Bet. 1 and 2	7
0.5 cc. salt + 0.5 cc. diglycin.....	0	Bet. 8 and 9	6	10	Bet. 0 and 1	6
1 cc. diglycin.....	0	8	4	10	Bet. 0 and 1	5
0.9 cc. salt + 0.1 cc. phenylglycocoll.....	Bet. 3 and 4	10	Bet. 7 and 8	10	Bet. 2	7
0.5 cc. salt + 0.5 cc. phenylglycocoll.....	5	10	Bet. 7 and 8	10	3	Bet. 7 and 8
1 cc. phenylglycocoll.....	8	10	Bet. 9	10	Bet. 4 and 5	10
0.9 cc. salt + 0.1 cc. glycyltryptophan.....	4	10	7	10	Bet. 1 and 2	7
0.5 cc. salt + 0.5 cc. glycyltryptophan.....	Bet. 1 and 2	10	7	10	Bet. 1 and 2	7
1 cc. glycyltryptophan.....	Bet. 1 and 2	10	6	10	1	7
0.9 cc. salt + 0.1 cc. leucin.....	3	10	7	10	Bet. 2 and 3	8
0.5 cc. salt + 0.5 cc. leucin.....	4	10	Bet. 7 and 8	10	Bet. 2 and 3	8
1 cc. leucin.....	Bet. 6 and 7	10	7	10	Bet. 2 and 3	8
0.9 cc. salt + 0.1 cc. alanin.....	Bet. 1 and 2	10	8	10	Bet. 1 and 2	7
0.5 cc. salt + 0.5 cc. alanin.....	Bet. 1 and 2	10	7	10	Bet. 1 and 2	7
1 cc. alanin.....	Bet. 1 and 2	10	7	10	Bet. 1 and 2	7
0.9 cc. salt + 0.1 cc. heteroalbumose.....	2	10	Bet. 6 and 7	10	Bet. 1 and 2	Bet. 6 and 7
0.5 cc. salt + 0.5 cc. heteroalbumose.....	Bet. 0 and 1	10	6	10	2	6
1 cc. heteroalbumose.....	Bet. 0	10	2	Bet. 9 and 10	1	6
0.9 cc. salt + 0.1 cc. protoalbumose.....	2	10	Bet. 0 and 1	7	Bet. 0 and 1	Bet. 5 and 6
0.5 cc. salt + 0.5 cc. protoalbumose.....	4	10	Bet. 7 and 8	10	2	7
1 cc. protoalbumose.....	Bet. 6 and 7	10	Bet. 8 and 9	10	2	7

en a accru l'intensité) tant de phénylglycocolle a été plus considérable.

La leucine a agi de la même façon.

La protoalbumose a accéléré l'hémolyse. La dose la plus forte a eu les effets les plus prononcés, sauf dans l'expérience 6 en présence de sérum inactivé, où l'on n'a pas constaté de différences entre les trois doses envisagées.

Le glycytryptophane a exercé, en l'absence de sérum, une légère action favorable dans les expériences 5 et 6 sur l'hémolyse, défavorable au contraire dans l'expérience 4, surtout à la dose forte. En présence de sérum frais, la forte dose a retardé légèrement le début de l'hémolyse, tandis que les deux autres n'ont exercé aucune action sur ce phénomène. En présence de sérum inactivé, la forte dose a quelque peu accéléré le début de l'hémolyse, tandis que les deux autres n'ont en aucune façon influencé ce phénomène.

En l'absence de sérum, le glycocolle a eu une action légèrement favorable sur l'hémolyse dans les expériences 4 et 6 à toutes les doses. Dans l'expérience 5, seule la dose la plus faible a quelque peu agi dans ce sens, tandis que les doses moyenne et forte n'ont exercé aucune action sur l'hémolyse. En présence de sérum frais ou inactivé, le glycocolle n'a exercé aucun effet sur l'hémolyse.

La diglycine, la triglycine, le tétraglycine et la pentaglycine retardent dans toutes les expériences le début de l'hémolyse et en diminuent souvent l'intensité. La dose la plus forte est toujours la plus efficace. En présence de sérum frais ou inactivé, cette action nocive persiste pour la diglycine et pour la triglycine; elle n'a pas été recherchée pour les deux autres peptides.

En l'absence de sérum, la forte dose d'alanine a légèrement accéléré le début de l'hémolyse dans l'expérience 5. L'alanine a eu la même action, aux trois doses utilisées, dans l'expérience 4, et cela surtout à la forte dose. Dans l'expérience 6, cet acide aminé n'a exercé, aux trois doses expérimentées, aucune action sur l'hémolyse. En présence de sérum frais, dans cette expérience, on a observé un très léger retard du début de l'hémolyse

avec la forte dose. En présence de sérum inactivé, cette même dose a amené une très légère diminution d'intensité de l'hémolyse.

L'hétéroalbumose a exercé des effets défavorable sur l'hémolyse, tant en l'absence de sérum qu'en présence de sérum frais ou inactivé de cobaye, dans les expériences 4 et 6 et cela surtout à la forte dose. Ces effets nocifs sont même plutôt accentués par la présence de sérum pour ce qui concerne l'intensité de l'hémolyse. Dans l'expérience 5, en l'absence de sérum, la forte dose d'hétéroalbumose accélère le début de l'hémolyse, tandis que les doses moyenne et forte retardent l'apparition de ce phénomène et en diminuent l'intensité.

Experiments with red corpuscles of dog

Nous ne disposons que de deux expériences effectuées avec des hématies de chien.

Comme chez le cobaye et comme chez l'homme, le venin de cobra est parvenu, chez le chien, à provoquer l'hémolyse en l'absence de sérum de cobaye. L'addition de ce sérum a retardé l'hémolyse dans l'expérience 7 et l'a au contraire accélérée dans l'expérience 8. Le sérum chauffé au préalable à 56 degrés a retardé l'hémolyse et en a diminué l'intensité.

Le phénylglycocolle a accéléré, en l'absence de sérum, le processus hémolytique. En présence de sérum frais de cobaye, le phénylglycocolle a par contre retardé l'hémolyse. En présence de sérum inactivé, le phénylglycocolle a à la fois accéléré l'hémolyse et accru l'intensité du phénomène. Dans tous les cas, la dose la plus considérable de cet acide aminé, a présenté le maximum d'efficacité.

En l'absence de sérum, la leucine a accéléré notablement l'hémolyse. En présence de sérum frais de cobaye, l'accélération de l'hémolyse a persisté, surtout à la dose moyenne, dans l'expérience 7, tandis qu'on n'a décelé aucun effet de l'addition de cet acide aminé dans l'expérience 8, où l'hémolyse était déjà complète au bout de 2 heures dans le tube témoin. En présence de sérum inactivé, la leucine a accéléré l'hémolyse et accru l'intensité du phénomène surtout à la dose faible.

Experiment 7

EACH TEST TUBE CONTAINS 0.1 CC. OF 4 PER CENT SOLUTION OF COBALTINOM AND 1 DROP OF SUSPENSION OF RED CORPUSCLES OF DOG AND FURTHER	AND NO GUINEA PIG'S SERUM		AND 0.1 CC. OF GUINEA PIG'S SERUM	
	Intensity of hemolysis after		Intensity of hemolysis after	
	2 hours	19 hours	2 hours	19 hours
1 cc. salt.....	7	10	Between 2 and 3	10
0.9 cc. salt + 0.1 cc. glycoll.....	7	10	3	10
0.5 cc. salt + 0.5 cc. glycoll.....	6	10	3	10
1 cc. glycoll.....	6	10	2	10
0.9 cc. salt + 0.1 cc. diglycin.....	6	10	2	10
0.5 cc. salt + 0.5 cc. diglycin.....	0	8	2	10
1 cc. diglycin.....	0	8	1	10
0.9 cc. salt + 0.1 cc. triglycin.....	0	8	2	10
0.5 cc. salt + 0.5 cc. triglycin.....	0	Between 5 and 6	2	10
1 cc. triglycin.....	0	2	1	10
0.9 cc. salt + 0.1 cc. glycyltryptophan.....	Between 1 and 2	10	Between 3 and 4	10
0.5 cc. salt + 0.5 cc. glycyltryptophan.....	4	10	Between 3 and 4	10
1 cc. glycyltryptophan.....	3	10	3	10
0.9 cc. salt + 0.1 cc. leucin.....	10	10	5	10
0.5 cc. salt + 0.5 cc. leucin.....	10	10	7	10
1 cc. leucin.....	10	10	Between 3 and 4	10
0.9 cc. salt + 0.1 cc. leucylglycin.....	Between 2 and 3	8	3	10
0.5 cc. salt + 0.5 cc. leucylglycin.....	Between 2 and 3	7	3	10
1 cc. leucylglycin.....	3	4	3	10
0.9 cc. salt + 0.1 cc. protoalbumose.....	7	10	3	9
0.5 cc. salt + 0.5 cc. protoalbumose.....	7	10	3	9
1 cc. protoalbumose.....	Between 1 and 2	10	3	9

Experiment 8

EACH TEST TUBE CONTAINS 0.1 CC. OF 4 PER CENT SOLUTION OF COBRAVENOM AND 1 DROP OF RED CORPUSCLES OF DOG AND FURTHER:	AND NO GUINEA PIG'S SERUM		AND 0.1 CC. OF GUINEA PIG'S SERUM		AND 0.1 CC. OF PREVIOUSLY IN- ACTIVATED GUINEA PIG'S SERUM	
	Intensity of hemolysis after		Intensity of hemolysis after		Intensity of hemolysis after	
	2 hours	19 hours	2 hours	19 hours	2 hours	19 hours
1 cc. salt.....	Bet. 4 and 5	10	10	10	2	Bet. 7 and 8
0.9 cc. salt + 0.1 cc. glycocoll.....	Bet. 5 and 6	10	10	10	2	Bet. 7 and 8
0.5 cc. salt + 0.5 cc. glycocoll.....	Bet. 3 and 4	10	10	10	2	Bet. 7 and 8
1 cc. glycocoll.....	Bet. 3 and 4	10	10	10	2	Bet. 6 and 7
0.9 cc. salt + 0.1 cc. diglycin.....	Bet. 3	9	7	10	2	6
0.5 cc. salt + 0.5 cc. diglycin.....	Bet. 1 and 2	9	7	10	2	6
1 cc. diglycin.....	0	7	6	10	Bet. 0 and 1	5
0.9 cc. salt + 0.1 cc. triglycin.....	1	8	8	10	2	7
0.5 cc. salt + 0.5 cc. triglycin.....	0	6	6	10	Bet. 1 and 2	5
1 cc. triglycin.....	0	3	5	10	Bet. 0 and 1	3
0.9 cc. salt + 0.1 cc. phenylglycocoll.....	5	10	Bet. 9 and 10	10	4	Bet. 9 and 10
0.5 cc. salt + 0.5 cc. phenylglycocoll.....	8	10	Bet. 8 and 9	10	6	10
1 cc. phenylglycocoll.....	10	10	8	10	6	10
0.9 cc. salt + 0.1 cc. glycyltryptophan.....	Bet. 1 and 2	9	9	10	4	Bet. 8 and 9
0.5 cc. salt + 0.5 cc. glycyltryptophan.....	Bet. 1 and 2	9	9	10	3	Bet. 8 and 9
1 cc. glycyltryptophan.....	3	10	10	10	2	Bet. 8 and 9
0.9 cc. salt + 0.1 cc. leucin.....	7	10	10	10	7	10
0.5 cc. salt + 0.5 cc. leucin.....	7	10	10	10	6	10
1 cc. leucin.....	8	10	10	10	6	10
0.9 cc. salt + 0.1 cc. leucylglycin.....	3	9	10	10	5	9
0.5 cc. salt + 0.5 cc. leucylglycin.....	3	9	9	10	5	9
1 cc. leucylglycin.....	3	7	7	10	5	9
0.9 cc. salt + 0.1 cc. alanin.....	3	10	10	10	2	8
0.5 cc. salt + 0.5 cc. alanin.....	Bet. 2 and 3	10	10	10	2	8
1 cc. alanin.....	3	10	10	10	2	8
0.9 cc. salt + 0.1 cc. heteroalbumose.....	Bet. 4 and 5	10	7	10	1	Bet. 6 and 7
0.5 cc. salt + 0.5 cc. heteroalbumose.....	Bet. 4	10	Bet. 3 and 4	Bet. 8 and 9	2	5
1 cc. heteroalbumose.....	4	10	2	7	Bet. 0 and 1	4
0.9 cc. salt + 0.1 cc. protoalbumose.....	Bet. 4	10	10	10	2	8
0.5 cc. salt + 0.5 cc. protoalbumose.....	3	10	8	10	2	8
1 cc. protoalbumose.....	Bet. 0 and 1	8	8	10	2	6

Les diverses doses utilisées de glycytryptophane, mais surtout la dose la plus faible, ont, en l'absence de sérum, retardé l'hémolyse. À la dose moyenne et à la dose faible, ce peptide a, en outre, diminué l'intensité du processus hémolytique dans l'expérience 8. En présence de sérum frais de cobaye, les doses faible et moyenne de glycytryptophane ont légèrement accéléré l'hémolyse dans l'expérience 7 et l'ont par contre quelque peu retardée dans l'expérience 8. En présence de sérum inactivé, les doses faible et moyenne de ce peptide ont accéléré l'hémolyse et les trois doses expérimentées ont augmenté l'intensité du phénomène; la dose la plus faible a présenté le plus d'efficacité.

En l'absence de sérum, l'alanine a retardé le processus hémolytique. En présence de sérum frais de cobaye, elle n'a exercé aucune influence sur l'hémolyse. En présence de sérum inactivé, la forte dose d'alanine a retardé le début du phénomène et les trois doses expérimentées en ont accru quelque peu l'intensité.

Le glyocolle n'a exercé que des effets peu marqués sur l'hémolyse des hématies de chien par le venin de cobra. En l'absence de sérum, les doses moyenne et forte ont légèrement retardé l'hémolyse. La faible dose a quelque peu accéléré ce phénomène dans l'expérience 8 et est restée sans action dans l'expérience 7. En présence de sérum, dans l'expérience 7, les doses faible et moyenne ont légèrement accéléré le processus hémolytique, tandis que la forte dose l'a quelque peu retardé. Dans l'expérience 8, on n'a pu observer aucune influence du glyocolle sur l'hémolyse en présence de sérum frais de cobaye. Dans cette même expérience, en présence de sérum inactivé, la forte dose de glyocolle a légèrement diminué l'intensité de l'hémolyse.

Tant en l'absence de sérum qu'en présence de sérum inactivé, la diglycine et surtout la triglycine ont retardé l'hémolyse et diminué l'intensité de ce phénomène, et cela d'autant plus que la dose de peptide employée a été plus considérable. En présence de sérum frais de cobaye, on n'a plus observé que le retard du processus hémolytique.

La leucylglycine a, en l'absence de sérum, retardé l'hémolyse et diminué l'intensité de ce phénomène, et cela d'autant plus que la dose de peptide utilisée a été plus élevée. En présence

de sérum neuf de cobaye, la leucylglycine a légèrement accéléré le processus hémolytique, mais en a diminué l'intensité dans l'expérience 7. Elle a retardé, à la dose moyenne et surtout à la forte dose, la marche de l'hémolyse sans amoindrir l'intensité de ce phénomène dans l'expérience 8. En présence de sérum inactivé, la leucylglycine a accéléré l'hémolyse et en a accru l'intensité.

En l'absence de sérum, la protoalbumose a retardé l'hémolyse, et ceci surtout lorsqu'on a employé la forte dose de cette protéose. En présence de sérum frais de cobaye, le processus hémolytique a été légèrement accéléré, mais a décréu en intensité dans l'expérience 7. Il a manifesté dans ces conditions, dans l'expérience 8, aux doses moyenne et forte de protoalbumose, quelque retard par rapport au tube témoin sans que la présence de la protéose ait empêché l'hémolyse de finir par être complète. En présence de sérum inactivé, l'intensité de l'hémolyse a montré un léger accroissement lorsqu'on s'est servi des doses faible et moyenne de protoalbumose et a au contraire quelque peu diminué quand on a employé la forte dose de cette protéide.

Les doses moyenne et forte d'hétéroalbumose ont, en l'absence de sérum, amené un léger retard de l'hémolyse sans agir sur l'intensité finale du processus. Ce retard s'est montré, en présence de sérum frais de cobaye, aux trois doses d'hétéroalbumose employées. Il a été d'autant plus considérable que la quantité de cette protéose était plus grande. L'intensité de l'hémolyse a diminué dans le tube renfermant la dose moyenne et surtout dans celui contenant la forte dose d'hétéroalbumose. Les effets de cette protéose sont restés les mêmes en présence de sérum inactivé que de sérum frais; toutefois la faible dose de cette protéose n'a pas entraîné dans ce cas de diminution de l'intensité de l'hémolyse au bout de 2 heures, mais bien au bout de 19 heures.

Experiments with red corpuscles of rabbit

Ces recherches comprennent deux expériences dans lesquelles on a employé du sérum de lapin et une où l'on s'est servi de sérum de cobaye.

Experiment 9

EACH TEST TUBE CONTAINS 0.1 CC. OF 4 PER CENT SOLUTION OF COBRAVENOM AND 1 DROP OF SUSPENSION OF RED CORPUSCLES OF RABBIT AND FURTHER:	AND NO RABBIT'S SERUM		AND 0.1 CC. OF RABBIT'S SERUM		AND 0.1 CC. OF PREVIOUSLY INACTIVATED RABBIT'S SERUM	
	Intensity of hemolysis after		Intensity of hemolysis after		Intensity of hemolysis after	
	2½ hours	18 hours	2½ hours	18 hours	2½ hours	18 hours
1 cc. salt.....	0	Bet. 0 and 1	1	2	2	Bet. 2 and 3
0.9 cc. salt + 0.1 cc. glycocoll.....	0	Bet. 0 and 1	1	2	2	Bet. 2 and 3
0.5 cc. salt + 0.5 cc. glycocoll.....	0	Bet. 0 and 1	1	2	2	
1 cc. glycocoll.....	0	Bet. 0 and 1	1	1	1	Bet. 1 and 2
0.9 cc. salt + 0.1 cc. diglycin.....	0	Bet. 0 and 1	1	1	2	Bet. 1 and 2
0.5 cc. salt + 0.5 cc. diglycin.....	0	Bet. 0 and 1	0	Bet. 0 and 1	2	Bet. 1 and 2
1 cc. diglycin.....	0	0	0	2	2	2
0.9 cc. salt + 0.1 cc. triglycin.....	0	Bet. 0 and 1	1	2	1	1
0.5 cc. salt + 0.5 cc. triglycin.....	0	0	0	Bet. 0 and 1	1	1
1 cc. triglycin.....	0	0	0	Bet. 0 and 1	1	1
0.9 cc. salt + 0.1 cc. glycyltryptophan.....	0	0	1	1	3	4
0.5 cc. salt + 0.5 cc. glycyltryptophan.....	0	Bet. 1 and 2	0	1	1	Bet. 2 and 3
1 cc. glycyltryptophan.....	0	1	0	1	1	Bet. 2 and 3
0.9 cc. + 0.1 cc. leucin.....	0	3	1	4	3	4
0.5 cc. salt + 0.5 cc. leucin.....	1	5	1	6	3	6
1 cc. leucin.....	2	Bet. 7 and 8	2	7	4	6
0.9 cc. salt + 0.1 cc. leucylglycin.....	0	Bet. 0 and 1	1	Bet. 1 and 2	2	3
0.5 cc. salt + 0.5 cc. leucylglycin.....	0	Bet. 0 and 1	0	1	2	2
1 cc. leucylglycin.....	0	Bet. 0 and 1	0	Bet. 0 and 1	2	2
0.9 cc. salt + 0.1 cc. alanin.....	0	Bet. 0 and 1	1	2	2	Bet. 4 and 5
0.5 cc. salt + 0.5 cc. alanin.....	0	1	1	3	2	4
1 cc. alanin.....	0	Bet. 1 and 2	1	3	2	4
0.9 cc. salt + 0.1 cc. heteroalbumose.....	0	0	0	Bet. 0 and 1	1	Bet. 2 and 3
0.5 cc. salt + 0.5 cc. heteroalbumose.....	0	0	0	Bet. 0 and 1	1	Bet. 2 and 3
1 cc. heteroalbumose.....	0	0	Bet. 6 and 7	10	7	Bet. 8 and 9
0.9 cc. salt + 0.1 cc. protoalbumose.....	0	Bet. 0 and 1	1	2	2	3
0.5 cc. salt + 0.5 cc. protoalbumose.....	0	Bet. 0 and 1	1	2	2	2
1 cc. protoalbumose.....	2	5	1	2	2	2

Experiment 10

EACH TEST TUBE CONTAINS 0.1 CC. OF 4 PER CENT OF COBRA- VENOM AND 1 DROP OF SUSPENSION OF RED COR- PUSCLES OF RABBIT AND FURTHER:	AND NO RABBIT'S SERUM		AND 0.1 CC. OF RABBIT'S SERUM		AND 0.1 CC. OF PREVIOUSLY IN- ACTIVATED RABBIT'S SERUM	
	Intensity of hemolysis after		Intensity of hemolysis after		Intensity of hemolysis after	
	3 hours	19 hours	3 hours	19 hours	3 hours	19 hours
1 cc. salt.....	0	1	Bet. 1 and 2	4	Bet. 1 and 2	4
0.9 cc. salt + 0.1 cc. glycecoll.....	0	1	Bet. 1 and 2	3	Bet. 1 and 2	4
0.5 cc. salt + 0.5 cc. glycecoll.....	0	Bet. 0 and 1	Bet. 1 and 2	3	Bet. 1 and 2	3
1 cc. glycecoll.....	0	0	Bet. 1 and 2	Bet. 1 and 2	1	3
0.9 cc. salt + 0.1 cc. diglycin.....	0	Bet. 0 and 1	Bet. 1 and 2	2	Bet. 1 and 2	4
0.5 cc. salt + 0.5 cc. diglycin.....	0	0	1	Bet. 1 and 2	1	2
1 cc. diglycin.....	0	0	0	Bet. 0 and 1	1	Bet. 1 and 2
0.9 cc. salt + 0.1 cc. triglycin.....	0	Bet. 0 and 1	1	1	1	3
0.5 cc. salt + 0.5 cc. triglycin.....	0	0	0	Bet. 0 and 1	1	Bet. 1 and 2
1 cc. triglycin.....	0	0	0	Bet. 0 and 1	1	Bet. 0 and 1
0.9 cc. salt + 0.1 cc. glycyltrptophan.....	0	Bet. 0 and 1	1	Bet. 1 and 2	Bet. 1 and 2	6
0.5 cc. salt + 0.5 cc. glycyltrptophan.....	0	5	Bet. 0 and 1	Bet. 0 and 1	1	4
1 cc. glycyltrptophan.....	0	10	Bet. 0 and 1	Bet. 0 and 1	1	Bet. 2 and 3
0.9 cc. salt + 0.1 cc. leucin.....	0	5	1	4	Bet. 1 and 2	5
0.5 cc. salt + 0.5 cc. leucin.....	1	10	2	6	Bet. 2 and 3	7
1 cc. leucin.....	Bet. 1 and 2	10	3	7	4	Bet. 6 and 7
0.9 cc. salt + 0.1 cc. leucylglycin.....	0	1	Bet. 1 and 2	3	Bet. 1 and 2	3
0.5 cc. salt + 0.5 cc. leucylglycin.....	0	1	1	3	Bet. 1 and 2	3
1 cc. leucylglycin.....	0	1	1	2	1	2
0.9 cc. salt + 0.1 cc. alanin.....	0	2	2	4	1	4
0.5 cc. salt + 0.5 cc. alanin.....	0	2	1	5	1	5
1 cc. alanin.....	0	2	1	4	0	1
0.9 cc. salt + 0.1 cc. protoalbumose.....	0	1	1	4	Bet. 1 and 2	4
0.5 cc. salt + 0.5 cc. protoalbumose.....	0	3	1	4	Bet. 1 and 2	Bet. 3 and 4
1 cc. protoalbumose.....	0	5	1	4	Bet. 1 and 2	7

Experiment 11

EACH TEST TUBE CONTAINS 0.1 CC. OF 4 PER CENT SOLUTION OF COBRAVENOM AND 1 DROP OF SUSPENSION OF RED CORPUSCLES OF RABBIT AND FURTHER:	AND NO GUINEA PIG'S SERUM		AND 0.1 CC. OF GUINEA PIG'S SERUM		AND 0.1 CC. OF PREVIOUSLY INACTIVATED GUINEA PIG'S SERUM	
	Intensity of hemolysis after		Intensity of hemolysis after		Intensity of hemolysis after	
	1½ hours	18 hours	1½ hours	18 hours	1½ hours	18 hours
1 cc. salt.....	0	Bet. 0 and 1	7	10	0	Bet. 0 and 1
0.9 cc. salt + 0.1 cc. glycocoll.....	0	Bet. 1 and 2	7	10	0	Bet. 0 and 1
0.5 cc. salt + 0.5 cc. glycocoll.....	0	1	7	10	0	Bet. 0 and 1
1 cc. glycocoll.....	0	1	6	10	0	Bet. 0 and 1
0.9 cc. salt + 0.1 cc. diglycin.....	0	Bet. 0 and 1	7	10	0	Bet. 0 and 1
0.5 cc. salt + 0.5 cc. diglycin.....	0	Bet. 0 and 1	6	10	0	Bet. 0 and 1
1 cc. diglycin.....	0	Bet. 0 and 1	5	10	0	Bet. 0 and 1
0.9 cc. salt + 0.1 cc. phenylglycocoll.....	0	Bet. 1	6	10	0	Bet. 0 and 1
0.5 cc. salt + 0.5 cc. phenylglycocoll.....	0	1	4	7	0	1
1 cc. phenylglycocoll.....	Bet. 1 and 2	4	2	5	0	Bet. 1 and 2
0.9 cc. salt + 0.1 cc. glycyltryptophan.....	0	Bet. 1 and 2	7	10	0	Bet. 1 and 2
0.5 cc. salt + 0.5 cc. glycyltryptophan.....	0	3	7	10	0	Bet. 1 and 2
1 cc. glycyltryptophan.....	0	4	6	10	0	2
0.9 cc. salt + 0.1 cc. leucin.....	0	3	Bet. 6 and 7	10	0	2
0.5 cc. salt + 0.5 cc. leucin.....	Bet. 1 and 2	4	6	10	0	2
1 cc. leucin.....	Bet. 1 and 2	5	6	10	0	Bet. 2 and 3
0.9 cc. salt + 0.1 cc. alanin.....	0	Bet. 0 and 1	7	10	0	Bet. 0 and 1
0.5 cc. salt + 0.5 cc. alanin.....	0	Bet. 1 and 2	7	10	0	Bet. 1 and 2
1 cc. alanin.....	0	Bet. 1 and 2	7	10	0	Bet. 1 and 2
0.9 cc. salt + 0.1 cc. heteroalbumose.....	0	Bet. 1 and 2	5	8	0	Bet. 1 and 2
0.5 cc. salt + 0.5 cc. heteroalbumose.....	0	Bet. 1 and 2	3	5	0	Bet. 1 and 2
1 cc. heteroalbumose.....	0	Bet. 1 and 2	Bet. 0 and 1	Bet. 2 and 3	0	1
0.9 cc. salt + 0.1 cc. protoalbumose.....	0	Bet. 0 and 1	7	10	0	Bet. 0 and 1
0.5 cc. salt + 0.5 cc. protoalbumose.....	0	Bet. 0 and 1	7	10	0	Bet. 0 and 1
1 cc. protoalbumose.....	0	Bet. 0 and 1	6	10	0	Bet. 0 and 1

En l'absence de sérum, le venin de cobra est pour ainsi dire dénué de toute action de toute action hémolytique sur les globules rouges de lapin. On n'observe, en effet, dans les expériences 9 à 11 que des traces d'hémolyse au bout de 18 à 19 heures. L'addition de sérum frais de lapin accélère notablement le phénomène, qui devient plus intense au bout de $2\frac{1}{2}$ à 3 heures dans ces circonstances qu'il ne l'était au bout de 18 à 19 heures dans les tubes sans sérum. Mais, même au bout de 18 à 19 heures, l'hémolyse n'est pas très considérable dans ces conditions. Au contraire, l'addition de sérum frais de cobaye amène une hémolyse complète, dont le degré d'intensité est déjà fort accentué après 1 heure $\frac{3}{4}$ seulement.

Si l'on porte au préalable le sérum à 56 degrés, cela ne modifie pas l'action du sérum de lapin (expérience 10) ou cela l'améliore même quelque peu (expérience 9), tandis que cela enlève par contre toute efficacité au sérum de cobaye (expérience 11).

La leucine exerce une action très favorable sur l'hémolyse des globules de lapin par le venin de cobra, et cela d'autant plus que la quantité de cet acide aminé est plus grande. L'hémolyse peut même devenir complète en présence de 0.5 à un centimètre cube de leucine (expérience 10). La forte dose de glycyltryptophane permet parfois aussi (expérience 10) l'hémolyse complète en l'absence de sérum. Ce peptide est néanmoins moins efficace que la leucine. L'hémolyse survient d'habitude moins vite et est moins accentuée en présence de glycyltryptophane que de leucine. La faible dose de ce peptide présente même parfois (expérience 10) une légère action inhibitrice. Dans deux expériences (9 et 10) sur trois, la protoalbumose a favorisé l'hémolyse, et cela à la dose moyenne et surtout à la forte dose, mais sans atteindre l'efficacité de la leucine. Tel a aussi été le cas du phénylglycocolle dans l'expérience 11. L'alanine a accentué quelque peu, parfois à toutes les doses (expérience 10), parfois seulement aux doses moyenne et forte (expérience 11), la faible hémolyse due au venin de cobra non additionné de sérum. On a observé la même chose en présence de glycocolle dans l'expérience 11, et cela surtout lorsqu'on a employé la faible dose de cet acide aminé. Dans l'expérience 11, le glyco-

colle n'a exercé aucun effet sur l'hémolyse des globules de lapin par le venin de cobra. Dans l'expérience 10, la forte dose de cet acide aminé a empêché l'hémolyse. La diglycine n'a pas agi sur l'hémolyse ou bien a empêché, à forte dose, ce phénomène. La triglycine l'a empêché aux doses moyenne et forte. La leucylglycine n'a aucunement modifié la faible hémolyse provoquée par le venin. L'hétéroalbumose a très légèrement favorisé l'hémolyse dans l'expérience 11; elle a au contraire empêché ce phénomène, aux doses faible et moyenne, dans l'expérience 11, où l'on a employé une solution d'hétéroalbumose, dont la neutralisation n'a malheureusement pas été parfaite.

En présence de sérum frais de lapin, l'action de la leucine, et surtout de la forte dose de cet acide aminé, est restée très nette sur l'hémolyse, tout en étant plutôt moins accentuée que dans les tubes analogues ne renfermant pas ce sérum. Dans l'expérience 11, l'addition d'un centimètre cube d'hétéroalbumose et de 0.1 centimètre cube de sérum frais de lapin a amené une hémolyse rapide et complète. On a observé une très légère action favorable de 0.5 à un centimètre cube de cet acide aminé dans l'expérience 10. Par contre, en présence de sérum frais de lapin, le glycytryptophane n'a plus exercé que des effets inhibiteurs et l'on n'a plus constaté la moindre action favorable de la protoalbumose. Dans l'ensemble, le sérum de lapin a accentué les effets inhibiteurs des dérivés de désintégration des protéines et a atténué ou fait disparaître leurs effets favorables sur l'hémolyse des globules de lapin par le venin de cobra.

Portons le sérum de lapin pendant 30 minutes à 56 degrés. Cela ne modifie guère son action associée à celle de l'un ou l'autre des dérivés étudiés des protéines. On observe des effets favorables de 0.1 centimètre cube de glycytryptophane et de 0.1 à un centimètre cube de leucine dans les expériences 9 et 10. La forte dose de protoalbumose a agi dans ce sens dans l'expérience 10, mais pas dans l'expérience 9. La forte dose d'hétéroalbumose a accéléré considérablement, en présence de sérum frais de lapin, l'hémolyse et a accéléré l'intensité de ce phénomène. L'alanine a accru l'intensité de l'hémolyse dans l'expérience 9 aux trois doses expérimentées, et seulement à la dose moyenne

dans l'expérience 10. Dans cette dernière expérience, la forte dose d'alanine a exercé un effet inhibiteur net en présence de sérum chauffé de lapin. Les effets inhibiteurs du glyocolle, de la diglycine et surtout de la triglycine persistent en présence du sérum inactivé du lapin. La leucylglycine est restée, sans action nette, comme en l'absence de sérum.

L'hémolyse ayant été, dans l'expérience 11, complète au bout de 18 heures en présence de sérum frais de cobaye, nous n'avons naturellement pu mettre en évidence que les effets inhibiteurs de l'addition des dérivés des protéines. L'hétéroalbumose et à un moindre degré le phénylglyocolle ont retardé la marche du processus hémolytique et en ont diminué l'intensité, et cela d'autant plus que la dose de protéose ou d'acide aminé a été plus considérable. On a observé un léger retard de l'hémolyse dans les tubes renfermant, en même temps que 0.1 centimètre cube de sérum frais de cobaye, soit un centimètre cube de glyocolle, soit 0.5 à un centimètre cube de diglycine, soit un centimètre cube de glycytryptophane, soit 0.1 à un centimètre cube de leucine, soit un centimètre cube de protoalbumose.

En présence de sérum inactivé de cobaye, la très faible hémolyse, observée après 18 heures dans le tube témoin (expérience 11) est légèrement accrue en intensité dans les tubes contenant soit 0.5 à un centimètre cube de phénylglyocolle, soit 0.1 à un centimètre cube de glycytryptophane, soit 0.1 à un centimètre cube de leucine, soit 0.5 à un centimètre cube d'alanine, soit 0.1 à un centimètre cube d'hétéroalbumose. Pour de phénylglyocolle, la glycytryptophane et la leucine, la dose la plus forte est la plus active ces produits se rangeant ainsi par ordre croissant d'action favorable. Pour l'hétéroalbumose, la forte dose est au contraire moins efficace que les deux autres. Les doses moyenne et forte d'alanine ont le même degré, très faible du reste, d'efficacité.

Experiments with red corpuscles of sheep

Nous n'avons fait que deux expériences avec les hématies de mouton.

Experiment 12

EACH TEST TUBE CONTAINS 0.1 CC. OF 4 PER CENT SOLUTION OF COBAYENOM AND 1 DROP OF SUSPENSION OF RED CORPUSCLES OF SHEEP AND FURTHER	AND NO GUINEA PIG'S SERUM		AND 0.1 CC. OF GUINEA PIG'S SERUM		AND 0.1 CC. OF PREVIOUSLY IN- ACTIVATED GUINEA PIG'S SERUM
	Intensity of hemolysis after		Intensity of hemolysis after		
	3½ hours	20 hours	3½ hours	20 hours	
1 cc. salt.....	0	0	Between 9 and 10	10	0
0.9 cc. salt + 0.1 cc. glycocoll.....	0	0	10	10	0
0.5 cc. salt + 0.5 cc. glycocoll.....	0	0	Between 9 and 10	10	0
1 cc. glycocoll.....	0	0	9	10	0
0.9 cc. salt + 0.1 cc. diglycin.....	0	0	Between 9 and 10	10	0
0.5 cc. salt + 0.5 cc. diglycin.....	0	0	Between 9 and 10	10	0
1 cc. diglycin.....	0	0	10	10	0
0.9 cc. salt + 0.1 cc. triglycin.....	0	0	10	10	0
0.5 cc. salt + 0.5 cc. triglycin.....	0	0	8	10	0
1 cc. triglycin.....	0	0	8	10	0
0.9 cc. salt + 0.1 cc. phenylglycocoll.....	0	0	9	10	0
0.5 cc. salt + 0.5 cc. phenylglycocoll.....	0	0	2	6	0
1 cc. phenylglycocoll.....	0	2	0	Between 3 and 4	0
0.9 cc. salt + 0.1 cc. glycyltryptophan.....	0	Between 0 and 1	10	10	0
0.5 cc. salt + 0.5 cc. glycyltryptophan.....	0	Between 0 and 1	9	10	0
1 cc. glycyltryptophan.....	0	0	9	10	0
0.9 cc. salt + 0.1 cc. leucin.....	0	1	10	10	0
0.5 cc. salt + 0.5 cc. leucin.....	0	Between 1 and 2	10	10	0
1 cc. leucin.....	0	Between 1 and 2	9	10	0
0.9 cc. salt + 0.1 cc. leucylglycin.....	0	1	Between 9 and 10	10	0
0.5 cc. salt + 0.5 cc. leucylglycin.....	0	Between 0 and 1	6	10	0
1 cc. leucylglycin.....	0	0	10	10	0
0.9 cc. salt + 0.1 cc. alanin.....	0	Between 0 and 1	10	10	0
0.5 cc. salt + 0.5 cc. alanin.....	0	Between 0 and 1	10	10	0
1 cc. alanin.....	0	Between 0 and 1	7	9	0
0.9 cc. salt + 0.1 cc. heteroalbumose.....	0	Between 0 and 1	Between 1 and 2	Between 3 and 4	0
0.5 cc. salt + 0.5 cc. heteroalbumose.....	0	Between 0 and 1	0	Between 1 and 2	0
1 cc. heteroalbumose.....	0	Between 0 and 1	10	10	0
0.9 cc. salt + 0.1 cc. protoalbumose.....	0	Between 0 and 1	Between 9 and 10	Between 9 and 10	0
0.5 cc. salt + 0.5 cc. protoalbumose.....	0	0	9	9	0
1 cc. protoalbumose.....	0	0	0	0	0

En l'absence de sérum ou en présence de sérum de cobaye chauffé au préalable à 56 degrés, on n'observe pas d'hémolyse des globules rouges de mouton par le venin de cobra. En présence de sérum frais de cobaye, l'hémolyse a eu lieu très vite et a été complète dans l'expérience 12; elle a été d'intensité très faible dans l'expérience 13.

On a constaté, dans l'expérience 12, en l'absence de sérum, au bout de 20 heures, des traces d'hémolyse dans les tubes renfermant soit 0.1 à 0.5 centimètre cube de glycytryptophane, soit un centimètre cube de leucylglycine, soit 0.5 à un centimètre cube d'alanine, soit 0.1 à un centimètre cube d'hétéroalbumose, soit 0.1 centimètre cube de protoalbumose. L'intensité du processus a atteint 10% de l'hémolyse totale en présence de 0.1 à 0.5 centimètre cube de leucylglycine ou de 0.1 centimètre cube de leucine, 10 à 20% en présence de 0.5 à un centimètre cube de leucine, 20% en présence d'un centimètre cube de phénylglycocolle, ce qui a été le maximum. Aucune hémolyse n'a été décelée dans les tubes contenant soit du glycocolle, soit de la diglycine, soit de la triglycine, soit 0.1 à 0.5 centimètre cube de phénylglycocolle, soit un centimètre cube de glycytryptophane, soit 0.1 centimètre cube d'alanine, soit 0.5 à un centimètre cube de protoalbumose.

Dans cette même expérience, la présence de sérum inactivé n'a permis l'hémolyse que dans le tube renfermant un centimètre cube de phénylglycocolle et l'intensité du processus a été exactement la même que dans le tube analogue ne renfermant pas de sérum.

Dans l'expérience 13, on n'a pas constaté d'hémolyse même après 18 heures, tant en l'absence de sérum qu'en présence de sérum inactivé de cobaye, quelles qu'aient été la quantité et la nature de la protéose (protoalbumose, hétéroalbumose) du peptide (diglycine, glycytryptophane, leucylglycine) ou de l'acide aminé (glycocolle, phénylglycocolle, leucine, alanine) ajouté au mélange d'hématies de mouton et de venin de cobra.

Voyons maintenant ce qui a lieu en présence de sérum frais de cobaye. Les choses ne se sont pas passées de façon tout à fait identique dans les expériences 12 et 13. Ceci tient assuré-

ment à la grande différence dans l'intensité de l'hémolyse constatée entre les tubes témoins renfermant le sérum frais de cobaye, les hématies de mouton et le venin de cobra dans ces deux expériences.

Dans l'expérience 12, l'hémolyse a été quelque peu accélérée par l'addition soit de 0.1 centimètre cube de glyocolle, soit de 0.1 centimètre cube de triglycine, soit de 0.1 centimètre cube de glycyltryptophane, soit de 0.1 à 0.5 centimètre cube de leucine, soit de 0.1 à un centimètre cube d'alanine, soit de 0.1 centimètre cube de protoalbumose. Elle a été légèrement retardée dans les tubes renfermant soit un centimètre cube de glyocolle, soit 0.5 à un centimètre cube glycyltryptophane, soit un centimètre cube de leucine, soit un centimètre cube de protoalbumose. Le retard du processus hémolytique a été plus accentué en présence de 0.5 à un centimètre cube de triglycine. L'hémolyse a été d'autant plus retardée que la quantité du produit ajouté a été plus considérable dans les tubes renfermant soit 0.1 à un centimètre cube de phénylglyocolle, soit 0.1 à un centimètre cube de d'hétéroalbumose, soit 0.5 à un centimètre cube de leucylglycine. L'hémolyse ayant été complète dans le tube témoin, nous n'avons pu déceler, dans cette expérience, que la diminution d'intensité du phénomène sous l'influence de l'addition soit de 0.5 à un centimètre cube de phénylglyocolle, soit de 0.1 à un centimètre cube d'hétéroalbumose, soit, bien qu'à un faible degré seulement, de 0.5 à un centimètre cube de protoalbumose. Dans ces trois cas, la dose la plus forte de l'acide aminé ou de la protéose a eu l'effet le plus défavorable sur l'hémolyse.

Dans l'expérience 13, le glyocolle, la diglycine et surtout la leucine ont accéléré l'hémolyse et en ont accru l'intensité, en présence de sérum frais de cobaye, de façon d'autant plus forte que la quantité d'acide aminé ou de peptide a été plus considérable. Les trois doses de protoalbumose, la dose moyenne et surtout la forte dose de glycyltryptophane, les doses faibles et moyenne de leucylglycine, la forte dose d'alanine ont aussi accéléré l'hémolyse et augmenté son intensité. La forte dose de leucylglycine a par contre retardé le processus hémolytique,

tout en accroissant quelque peu son intensité. La dose moyenne de phénylglycocolle a diminué l'intensité de l'hémolyse et la forte dose de cet acide aminé a empêché ce phénomène, même après 18 heures. Les doses faible et moyenne d'hétéroalbumose ont retardé le processus hémolytique et en ont notablement diminué l'intensité; la forte dose de cette protéose a empêché l'hémolyse.

Experiments with red corpuscles of calf

Nous avons effectué quatre expériences avec les hématies de veau.

Tant en l'absence de sérum qu'en présence de sérum inactivé de cobaye, il ne se produit jamais d'hémolyse des globules rouges

Experiment 13

EACH TEST TUBE CONTAINS 0.1 CC. OF 4 PER CENT SOLUTION OF COBRAVENOM, 0.1 CC. OF GUINEA PIG'S SERUM, 1 DROP OF SUSPENSION OF RED CORPUSCLES OF SHEEP, AND FURTHER	INTENSITY OF HEMOLYSIS AFTER	
	2½ hours	18 hours
1 cc. salt.....	Between 0 and 1	2
0.9 cc. salt + 0.1 cc. glycocoll.....	1	Between 2 and 3
0.5 cc. salt + 0.5 cc. glycocoll.....	2	3
1 cc. glycocoll.....	2	4
0.9 cc. salt + 0.1 cc. diglycin.....	Between 2 and 3	4
0.5 cc. salt + 0.5 cc. diglycin.....	Between 3 and 4	6
1 cc. diglycin.....	4	6
0.9 cc. salt + 0.1 cc. phenylglycocoll.....	Between 1 and 2	Between 3 and 4
0.5 cc. salt + 0.5 cc. phenylglycocoll.....	Between 0 and 1	1
1 cc. phenylglycocoll.....	0	0
0.9 cc. salt + 0.1 cc. glycyltryptophan...	Between 0 and 1	2
0.5 cc. salt + 0.5 cc. glycyltryptophan...	Between 1 and 2	3
1 cc. glycyltryptophan.....	2	4
0.9 cc. salt + 0.1 cc. leucin.....	1	Between 2 and 3
0.5 cc. salt + 0.5 cc. leucin.....	Between 1 and 2	5
1 cc. leucin.....	2	7
0.9 cc. salt + 0.1 cc. leucylglycin.....	Between 1 and 2	4
0.5 cc. salt + 0.5 cc. leucylglycin.....	0	4
1 cc. leucylglycin.....	0	Between 2 and 3
0.9 cc. salt + 0.1 cc. alanin.....	Between 0 and 1	2
0.5 cc. salt + 0.5 cc. alanin.....	Between 0 and 1	2
1 cc. alanin.....	Between 1 and 2	3
0.9 cc. salt + 0.1 cc. heteroalbumose....	0	Between 0 and 1
0.5 cc. salt + 0.5 cc. heteroalbumose....	0	Between 0 and 1
1 cc. heteroalbumose.....	0	0
0.9 cc. salt + 0.1 cc. protoalbumose....	2	3
0.5 cc. salt + 0.5 cc. protoalbumose....	2	3
1 cc. protoalbumose.....	1	3

No hemolysis in the several test tubes without guinea pig's serum, 0.1 cc. of previously inactivated guinea pig's serum.

Experiment 14

EACH TEST TUBE CONTAINS 0.1 CC. OF 4 PER CENT SOLUTION OF COBRAVENOM AND 1 DROP OF SUSPENSION OF RED CORPUSCLES OF CALF AND FURTHER	AND NO GUINEA PIG'S SERUM		AND 0.1 CC. OF GUINEA PIG'S SERUM		AND 0.1 CC. OF PREVIOUSLY IN- ACTIVATED GUINEA PIG'S SERUM
	Intensity of hemolysis after		Intensity of hemolysis after		
	18 hours		18 hours		
	2 hours	2 hours	2 hours	18 hours	
1 cc. salt	0	0	3	8	0
0.9 cc. salt + 0.1 cc. glycocoll	0	Between 0 and 1	Between 2 and 3	8	0
0.5 cc. salt + 0.5 cc. glycocoll	0	Between 0 and 1	2	8	0
1 cc. glycocoll	0	0	2	8	0
0.9 cc. salt + 0.1 cc. diglycin	0	0	Between 2 and 3	8	0
0.5 cc. salt + 0.5 cc. diglycin	0	0	Between 1 and 2	7	0
1 cc. diglycin	0	0	1	6	0
0.9 cc. salt + 0.1 cc. phenylglycocoll	0	0	3	Between 7 and 8	0
0.5 cc. salt + 0.5 cc. phenylglycocoll	0	0	Between 0 and 1	4	0
1 cc. phenylglycocoll	0	0	0	2	0
0.9 cc. salt + 0.1 cc. glycyltryptophan	0	Between 0 and 1	3	8	0
0.5 cc. salt + 0.5 cc. glycyltryptophan	0	Between 0 and 1	3	8	0
1 cc. glycyltryptophan	0	Between 0 and 1	3	Between 7 and 8	0
0.9 cc. salt + 0.1 cc. leucin	0	Between 0 and 1	3	8	0
0.5 cc. salt + 0.5 cc. leucin	0	Between 0 and 1	3	8	0
1 cc. leucin	0	Between 0 and 1	Between 1 and 2	Between 6 and 7	0
0.9 cc. salt + 0.1 cc. alanin	0	Between 0 and 1	3	8	0
0.5 cc. salt + 0.5 cc. alanin	0	Between 0 and 1	3	8	0
1 cc. alanin	0	Between 0 and 1	3	8	0
0.9 cc. salt + 0.1 cc. heteroalbumose	0	Between 0 and 1	Between 0 and 1	2	0
0.5 cc. salt + 0.5 cc. heteroalbumose	0	Between 0 and 1	0	0	0
1 cc. heteroalbumose	0	Between 0 and 1	0	0	0
0.9 cc. salt + 0.1 cc. protoalbumose	0	0	3	8	0
0.5 cc. salt + 0.5 cc. protoalbumose	0	0	3	8	0
1 cc. protoalbumose	0	0	3	8	0

Experiments 15 to 17

EACH TEST TUBE CONTAINS 0.1 CC. OF 4 PER CENT SOLUTION OF COBRAVENOM, 0.1 CC. OF GUINEA PIG'S SERUM, 1 DROP OF SUSPENSION OF RED CORPUSCLES OF CALF, AND FURTHER	EXPERIMENT 15 INTENSITY OF HEMOLYSIS AFTER		EXPERIMENT 16 INTENSITY OF HEMOLYSIS AFTER		EXPERIMENT 17 INTENSITY OF HEMOLYSIS AFTER	
	2 hours		18 hours		5 hours	
1 cc. salt.....	Bet. 6 and 7	10	2	8	9	10
0.9 cc. salt + 0.1 cc. glycocoll.....	Bet. 6	10	2	Bet. 9 and 10	9	10
0.5 cc. salt + 0.5 cc. glycocoll.....	Bet. 5 and 6	10	2	Bet. 9 and 10	9	10
1 cc. glycocoll.....	Bet. 5 and 6	10	2	10	9	10
0.9 cc. salt + 0.1 cc. diglycin.....	Bet. 5 and 6	10	2	10	8	10
0.5 cc. salt + 0.5 cc. diglycin.....	Bet. 4	10	2	10	7	9
1 cc. diglycin.....	3	7	2	8	6	8
0.9 cc. salt + 0.1 cc. triglycin.....			2	10	7	9
0.5 cc. salt + 0.5 cc. triglycin.....			2	10	6	8
1 cc. triglycin.....	Bet. 5 and 6	10	2	10	6	7
0.9 cc. salt + 0.1 cc. phenylglycocoll.....	Bet. 5 and 6	10	2			
0.5 cc. salt + 0.5 cc. phenylglycocoll.....	Bet. 5 and 6	10	2			
1 cc. phenylglycocoll.....	Bet. 5 and 6	10	2			
0.9 cc. salt + 0.1 cc. glycylytryptophan.....	Bet. 5 and 6	10	2			
0.5 cc. salt + 0.5 cc. glycylytryptophan.....	Bet. 5 and 6	10	2			
1 cc. glycylytryptophan.....	Bet. 5 and 6	10	2			
0.9 cc. salt + 0.1 cc. leucin.....	Bet. 5 and 6	10	2			
0.5 cc. salt + 0.5 cc. leucin.....	Bet. 5 and 6	10	2			
1 cc. leucin.....	Bet. 5 and 6	10	2			
0.9 cc. salt + 0.1 cc. alanin.....	Bet. 5 and 6	10	2			
0.5 cc. salt + 0.5 cc. alanin.....	Bet. 5 and 6	10	2			
1 cc. alanin.....	Bet. 5 and 6	10	2			
0.9 cc. salt + 0.1 cc. heteroalbumose.....	Bet. 5 and 6	10	2			
0.5 cc. salt + 0.5 cc. heteroalbumose.....	Bet. 5 and 6	10	2			
1 cc. heteroalbumose.....	Bet. 5 and 6	10	2			
0.9 cc. salt + 0.1 cc. protoalbumose.....	Bet. 5 and 6	10	2			
0.5 cc. salt + 0.5 cc. protoalbumose.....	Bet. 5 and 6	10	2			
1 cc. protoalbumose.....	Bet. 5 and 6	10	2			

No hemolysis in the several test tubes without guinea-pig's serum, or with 0.1 cc. of previously inactivated guinea-pig's serum.

de boeuf par le venin de cobra. Au contraire, en présence de sérum frais de cobaye, l'hémolyse est déjà nette au bout de deux heures; elle est très considérable et même complète après 18 à 20 heures.

L'addition de l'un ou l'autre dérive des protéines n'a pas amené la moindre hémolyse, en l'absence de sérum, dans trois expériences (15, 16 et 17) sur quatre. Dans l'expérience 14, on a, par contre, observé, dans ces conditions, des traces d'hémolyse, après 18 heures dans les tubes renfermant soit 0.1 à 0.5 centimètre cube de glycocolle, soit 0.1 à un centimètre cube de leucine, d'alanine, de glycytryptophane ou d'hétéroalbumose.

En présence de sérum inactivé, on n'a jamais constaté d'hémolyse, quel qu'ait été le peptide, la protéose ou l'acide aminé ajouté.

Les divers dérivés des protéines n'ont exercé d'effets d'avantages sur l'hémolyse, en présence de sérum frais de cobaye, que dans l'expérience 16. Le glycocolle a accru l'intensité de l'hémolyse, surtoit à la dose forte, bien qu'il ait retardé, aux doses moyennne et forte le début du phénomène. Les doses faible et moyenne de diglycine ont rendu l'hémolyse complète, tandis que la forte dose n'a pas modifié l'intensité de ce phénomène, bien que les trois doses de ce peptide aient retardé le processus hémolytique. Les trois doses de triglycine ont accéléré de façon notable l'hémolyse et ont permis son achèvement entre 2 et 19 heures. La leucine et le glycytryptophane ont accéléré, à la faible dose, l'hémolyse et l'ont rendue complète à toutes les doses. L'alanine a accru, surtout à la forte dose, l'intensité de l'hémolyse. Les doses faible et moyenne d'hétéroalbumose ont accéléré le processus hémolytique, mais l'intensité du phénomène n'a pas été modifiée par rapport au tube témoin lorsqu'on a employé la faible dose de cette protéose et a diminué sous l'influence de la dose moyenne. Il ne s'est pas produit d'hémolyse dans le tube renfermant la forte dose d'hétéroalbumose. Quant à la protoalbumose, elle s'est bornée, dans cette expérience, à retarder, aux doses moyenne et forte, le processus hémolytique sans en influencer l'intensité.

Dans les trois autres expériences, on n'a pu mettre en évidence,

en présence de sérum, frais de cobaye, que des retards de l'hémolyse et des diminutions plus ou moins accentuées de l'intensité de ce phénomène sous l'influence des protéoses, des peptides et des acides aminés étudiés. Cette action inhibitrice a été la plus accentuée pour l'hétéroalbumose qui est même parvenue à empêcher l'hémolyse, aux doses moyenne et forte dans l'expérience 14, et à la forte dose dans l'expérience 17. Le phénylglycocolle a exercé des effets inhibiteurs très notables, tant sur la marche que sur l'intensité de l'hémolyse, et cela d'autant plus fort qu'on a employé une dose plus considérable de cet acide aminé. Le glycocolle s'est borné à retarder le début de l'hémolyse dans les expériences 14 et 15 et n'a modifié en aucune façon le pouvoir hémolytique dans l'expérience 17. La diglycine a retardé davantage l'hémolyse que le glycocolle. Elle a exercé cette action à toutes les doses dans les expériences 14 et 15 à la dose moyenne et surtout à la forte dose dans l'expérience 17. Dans ces trois expériences, la forte dose de diglycine a diminué l'intensité de l'hémolyse, il en a été de même, bien qu'à un moindre degré, de la dose moyenne dans les expériences 14 et 17. La triglycine a retardé davantage l'hémolyse et en a diminué dans une plus forte proportion l'intensité que la diglycine; la forte dose de chacun de ces deux peptides a eu l'effet nocif le plus caractérisé. La forte dose de leucine, et parfois aussi les autres doses de cet acide aminé, ont retardé le processus hémolytique. La forte dose de leucine a, en outre, diminué l'intensité de l'hémolyse dans les expériences 14 to 17. L'alanine a retardé l'hémolyse à toutes les doses dans l'expérience 15 et seulement à la forte dose dans l'expérience 17, sans agir sur l'intensité de ce phénomène. Elle n'a montré aucune action dans l'expérience 14. La forte dose de glycytryptophane a diminué l'intensité de l'hémolyse dans les trois expériences 14, 15 et 17. Ce peptide, a, en outre, amené un retard dans la marche du phénomène, à toutes les doses, mais surtout à la forte dose, dans les expériences 15 et 17. La protoalbumose n'a rien modifié au processus hémolytique dans les expériences 14 et 17; elle en a retardé la marche à toutes les doses dans l'expérience 15.

Experiment 18

EACH TEST TUBE CONTAINS 0.2 c.1 OF 4 PER CENT SOLUTION OF COBRAVENOM AND 1 DROP OF SUSPENSION OF RED CORPUSCLES OF OX AND FURTHER:	AND NO GUINEA PIG'S SERUM		AND 0.1 CC. OF GUINEA PIG'S SERUM		AND 0.1 CC. OF PREVIOUSLY IN-ACTIVATED GUINEA PIG'S SERUM
	Intensity of hemolysis after		Intensity of hemolysis after		
	2½ hours	18 hours	2½ hours	18 hours	
1 cc. salt.....	0	0	Between 2 and 3	Between 7 and 8	0
0.9 cc. salt + 0.1 cc. glycocoll.....	0	0	2	Between 7 and 8	0
0.5 cc. salt + 0.5 cc. glycocoll.....	0	0	2	Between 6 and 7	0
1 cc. glycocoll.....	0	0	1	Between 6 and 7	0
0.9 cc. salt + 0.1 cc. diglycin.....	0	0	Between 1 and 2	7	0
0.5 cc. salt + 0.5 cc. diglycin.....	0	0	Between 0 and 1	Between 5 and 6	0
1 cc. diglycin.....	0	0	0	Between 3 and 4	0
0.9 cc. salt + 0.1 cc. phenylglycocoll.....	0	0	2	7	0
0.5 cc. salt + 0.5 cc. phenylglycocoll.....	0	0	Between 0 and 1	6	0
1 cc. phenylglycocoll.....	0	0	0	3	0
0.9 cc. salt + 0.1 cc. glycyltryptophan.....	0	0	Between 2 and 3	7	0
0.5 cc. salt + 0.5 cc. glycyltryptophan.....	0	0	Between 1 and 2	7	0
1 cc. glycyltryptophan.....	0	0	Between 0 and 1	Between 4 and 5	0
0.9 cc. salt + 0.1 cc. leucin.....	0	0	3	7	0
0.5 cc. salt + 0.5 cc. leucin.....	0	1	Between 2 and 3	7	0
1 cc. leucin.....	0	1	1	6	0
0.9 cc. salt + 0.1 cc. alanin.....	0	0	2	Between 7 and 8	0
0.5 cc. salt + 0.5 cc. alanin.....	0	1	2	7	0
1 cc. alanin.....	0	1	Between 1 and 2	7	0
0.9 cc. salt + 0.1 cc. heteroalbumose.....	0	0	Between 0 and 1	2	0
0.5 cc. salt + 0.5 cc. heteroalbumose.....	0	Between 0 and 1	0	Between 0 and 1	0
1 cc. heteroalbumose.....	0	Between 0 and 1	0	0	0
0.9 cc. + 0.1 cc. protoalbumose.....	0	0	3	8	0
0.5 cc. salt + 0.5 cc. protoalbumose.....	0	0	3	8	0
1 cc. protoalbumose.....	0	0	3	8	0

Experiments with red corpuscles of ox

Il nous reste à parler des deux expériences 18 et 19 effectuées avec des hématies de boeuf.

Tant en l'absence de sérum qu'en présence de sérum de cobaye, chauffé au préalable à 56 degrés, les hématies de boeuf n'ont pas subi d'hémolyse par le venin de cobra. En présence de sérum frais de cobaye, l'hémolyse est déjà très nette au bout d'une heure et demie; elle est très considérable ou même complète au bout de 18 heures.

Aucun des dérivés des protéines examiné n'est parvenu à amener de l'hémolyse, en l'absence de sérum, dans l'expérience 19. Il en a été de même du glycocolle, de la diglycine, du phénylglycocolle, du glycyltryptophane et de la protoalbumose dans l'expérience 18. On a constaté des traces d'hémolyse dans cette dernière expérience sous l'influence de 0.5 un centimètre

Experiment 19

EACH TEST TUBE CONTAINS 0.1 CC. OF 4 PER CENT SOLUTION OF COBRAVENOM, 0.1 CC. OF GUINEA PIG'S SERUM, 1 DROP OF SUSPENSION OF RED CORPUSCLES OF OX, AND FURTHER	INTENSITY OF HEMOLYSIS AFTER	
	1½ HOURS	18 hours
1 cc. salt.....	2	10
0.9 cc. salt + 0.1 cc. glycocoll.....	2	10
0.5 cc. salt + 0.5 cc. glycocoll.....	1	Between 8 and 9
1 cc. glycocoll.....	1	8
0.9 cc. salt + 0.1 cc. diglycin.....	2	Between 9 and 10
0.5 cc. salt + 0.5 cc. diglycin.....	Between 0 and 1	8
1 cc. diglycin.....	0	6
0.9 cc. salt + 0.1 cc. phenylglycocoll.....	Between 2 and 3	9
0.5 cc. salt + 0.5 cc. phenylglycocoll.....	Between 1 and 2	6
1 cc. phenylglycocoll.....	Between 0 and 1	3
0.9 cc. salt + 0.1 cc. glycyltryptophan...	Between 0 and 1	Between 9 and 10
0.5 cc. salt + 0.5 cc. glycyltryptophan...	Between 0 and 1	9
1 cc. glycyltryptophan.....	0	9
0.9 cc. salt + 0.1 cc. leucin.....	2	Between 9 and 10
0.5 cc. salt + 0.5 cc. leucin.....	Between 0 and 1	9
1 cc. leucin.....	0	8
0.9 cc. salt + 0.1 cc. alanin.....	2	10
0.5 cc. salt + 0.5 cc. alanin.....	2	10
1 cc. alanin.....	Between 1 and 2	10
0.9 cc. salt + 0.1 cc. heteroalbumose.....	0	4
0.5 cc. salt + 0.5 cc. heteroalbumose.....	0	2
1 cc. heteroalbumose.....	0	Between 0 and 1
0.9 cc. salt + 0.1 cc. protoalbumose.....	2	10
0.5 cc. salt + 0.5 cc. protoalbumose.....	2	10
1 cc. protoalbumose.....	2	10

No hemolysis in the several test tubes without guinea pig's serum or with 0.1 cc. of previously inactivated guinea pig's serum.

cube d'hétéroalbumose. L'hémolyse a atteint environ 10% de l'hémolyse totale dans cette même expérience dans les tubes renfermant 0.5 à un centimètre cube d'alanine ou de leucine.

En présence de sérum inactivé, il ne s'est jamais produit d'hémolyse, quel qu'ait été la protéose, le peptide ou l'acide aminé ajouté.

A part une insignifiante accélération du processus hémolytique par la faible dose de leucine et par les trois doses de protoalbumose dans l'expérience 18 et par la faible dose de phénylglycocolle dans l'expérience 19 ainsi qu'une très légère augmentation de l'intensité de l'hémolyse par la protoalbumose dans l'expérience 18, on n'a point décelé d'effets favorables de l'addition de l'un ou l'autre dérivé des protéines sur l'hémolyse des hématies de boeuf par le venin de cobra additionné de sérum frais de cobaye. La protoalbumose n'a exercé aucun effet sur l'hémolyse dans l'expérience 19. L'alanine n'a pas empêché l'hémolyse d'être complète au bout de 18 heures dans cette expérience, et il en a aussi été ainsi de 0.1 centimètre cube de glycocolle. L'addition de 0.1 centimètre cube d'alanine ou de glycocolle n'a pas modifié l'intensité de l'hémolyse dans l'expérience 18. Par contre, l'addition soit de 0.5 à un centimètre cube de glycocolle, soit de 0.1 à un centimètre cube de diglycine, de glycylothrophane, d'hétéroalbumose, de phénylglycocolle ou de leucine a amené, dans les expériences 18 et 19, une diminution dans l'intensité de l'hémolyse, d'autant plus considérable que la dose du dérivé protéique employé a été plus grande. En outre, dans l'expérience 18, 0.5 à un centimètre cube d'alanine a fait légèrement diminuer l'intensité de l'hémolyse. La forte dose d'hétéroalbumose a empêché l'hémolyse dans l'expérience 18 et n'a permis qu'une très faible et transitoire hémolyse dans l'expérience 19. Le processus hémolytique a été retardé dans presque tous ces cas, et cela en général le plus par la forte dose du produit considéré. Les effets inhibiteurs sont surtout marqués pour l'hétéroalbumose, puis viennent par ordre décroissant d'action nocive le phénylglycocolle, la diglycine, la leucine, le glycocolle, le glycylothrophane et l'alanine.

Dans l'ensemble, l'action des acides aminés, des peptides et

des protéoses sur l'hémolyse des globules rouge de boeuf par le venin de cobra ressemble beaucoup à celle exercée par ces mêmes produits sur l'hémolyse des globules rouges de veau, mais les effets inhibiteurs sont encore plus considérables vis-à-vis des hématies de boeuf que de celles de veau.

GENERAL CONSIDERATIONS

Le nombre d'expériences effectué avec les hématies des diverses espèces animales envisagées est trop peu considérable pour qu'on soit autorisé à tirer de nos expériences autre chose que des indications en vue de recherches futures. C'est pour cela qu'al nous paraît prématuré d'insister sur les diverses considérations qu'on pourrait aisément développer à propos de l'action des acides aminés, des peptides et des protéoses dans l'hémolyse. Nous aurons sans doute lieu d'y revenir à l'occasion de travaux ultérieurs. Il nous paraît cependant utile d'appeler dès à présent l'attention sur la possibilité de l'intervention des acides aminés, toujours présents dans le sang, dans les diverses réactions biologiques dans lesquelles l'hémolyse intervient.

On sait que la teneur du sang en acides aminés s'accroît après une saignée abondante et à un degré bien plus grand pendant la digestion d'un repas très riche en protéines. Quatre heures après l'ingestion de viande crue de boeuf, la teneur de sang carotidien en azote aminé est devenue plus du double du chiffre à l'état de jeûne (6). La teneur du sang en acides aminés varie aussi, chez l'homme, dans divers états pathologiques. C'est ainsi qu'on a constaté de l'hyperaminoacidémie pendant la cirrhose atrophique du foie accompagnée d'ictère et pendant les anémies posthémorragiques (7).

Or, dans nos recherches in vitro, le plus ou moins grand degré d'intensité des effets favorables ou défavorables de certains dérivés des protéines sur l'hémolyse dépend souvent de la teneur du mélange en l'acide aminé, le peptide ou la protéose ajouté à la suspension de globules rouges additionnée de venin de cobra. Il pourrait bien en être de même in vivo. Dès lors, des modifications de la teneur du sang en certains acides aminés ou en d'autres composés, formés lors du scindage des protéines, jouent

peut-être un rôle dans la réaction de Wassermann, dans le réaction d'activation du venin de cobra par le sérum de sujets atteints d'affections rénales (8) et dans d'autres réactions biologiques utilisées en clinique.

Il y a, par conséquent, fortes intérêt à poursuivre les investigations sur l'action des divers dérivés du scindage des protéines sur différents processus hémolytiques.

Nous résumons ci-dessous les données principales, qui paraissent ressortir de nos expériences et dont la vérification par le plus nombreuses recherches, s'impose.

SUMMARY

1. The effects of the amino-acids, peptids and proteoses upon the cobra venom hemolysis vary according to the species of blood corpuscles used. They depend also upon the amount of the respective compound present in the blood-venom mixture.

2. The hemolytic action of cobra venom on the red cells of guinea pig's blood is markedly hastened and increased in intensity by phenylglycocoll, leucin, glycyltryptophan, and protoalbumose. The hemolysis is produced more quickly in the presence of large quantities than it is with small amounts of phenylglycocoll and leucin. Heteroalbumose tends slightly to increase the intensity of the hemolysis; alanin, on the contrary, diminishes it a little. Glycocoll and particularly diglycin, triglycin and leucylglycin inhibit the hemolysis in direct proportion to their concentration.

In the presence of 0.1 cc. of fresh guinea pig's serum the accelerating action of protoalbumose and of glycyltryptophan and the inhibiting action of the strong concentrations of diglycin, of triglycin and of leucylglycin is hardly greater than that of the weaker concentrations. Heteroalbumose shows marked effects.

The presence of inactive serum exercises a protective action upon the blood corpuscles. It does not interfere with the acceleration of the hemolysis by phenylglycocoll, but it does diminish the adjuvant effects of glycyltryptophan and leucin and it almost completely annihilates those of protoalbumose.

It weakens considerably the marked effects of diglycin. Glycocol and heteroalbumose appear no longer to exercise any influence on the hemolysis.

3. The influence of the amino acids, peptids and proteoses upon human blood presents much resemblance to that exercised by these same products upon the blood of the guinea pig. In every instance the weak doses of glycocol accelerate very slightly the hemolysis of the human red corpuscles. Heteroalbumose often exercises an unfavorable effect on the hemolysis in the absence of serum. Glycyltryptophan has, according to the dose, sometimes a slightly favorable action, sometimes a slightly unfavorable one. Tetraglycin and pentaglycin hinder the hemolysis as does diglycin and triglycin.

4. Phenylglycocol and leucin favor the hemolysis of dog's blood by cobra venom in the absence of serum as well as in the presence of inactivated guinea pig's serum. The accelerating action of leucin persists in the presence of fresh guinea pig's serum while phenylglycocol on the contrary retards the hemolysis under these circumstances. The influence exerted by glycyltryptophan is unfavorable in the absence of serum, variable in the presence of fresh guinea pig's serum and favorable in the presence of inactivated serum. Alanin and glycocol have not a very marked action upon the hemolysis. In the absence of serum, the influence of diglycin, triglycin, leucylglycin, protoalbumose, and heteroalbumose is unfavorable to the hemolysis. The same is true of the action of diglycin, triglycin, and heteroalbumose in the presence of fresh or inactivated guinea pig's serum. In the presence of fresh guinea pig's serum variable effects are produced by leucylglycin and protoalbumose according to the dose and according to the suspension of the dog's blood. Leucylglycin favors the hemolysis in the presence of inactivated serum while protoalbumose exerts a variable influence upon that phenomenon under this condition.

5. The cobra venom hemolysis of rabbit's red corpuscles without the addition of serum is not marked excepting in the presence of leucin and sometimes also of glycyltryptophan, and of protoalbumose, the strongest dose of the preparation

examined being always the most effective. The addition of fresh guinea pig's serum elicits a very considerable inhibitory action on the part of heteroalbumose and phenylglycocoll. The protective action of the inactivated guinea pig's serum is very intense and greatly diminishes the favorable action of leucin and of glycytryptophan. The addition of fresh rabbit's serum diminishes the favorable effect of leucin and extinguishes completely that of glycytryptophan and of protoalbumose; it accentuates the inhibiting influence of the large doses of diglycin, triglycin and leucylglycin. Inactivated rabbit's serum hardly modifies the effects of the different amino acids, peptids and proteoses upon the cobra venom hemolysis of the red corpuscles of this animal species.

6. In the absence of fresh guinea-pig's serum, cobra venom, when combined with appropriate quantities of certain protein derivatives, produces only rarely, a very slight and slow hemolysis of the red corpuscles of the sheep, the calf and the ox. In the presence of inactivated guinea pig's serum hemolysis of the red corpuscles of the calf and the ox is never observed. The same is true also for the blood of the sheep excepting for one instance where the addition of one cubic centimeter of phenylglycocoll has caused the same degree of hemolysis as without the serum.

7. In the presence of fresh guinea pig's serum the influence of the same protein derivative upon the venom hemolysis of sheep's red corpuscles varies very greatly according to the amount of the substance used and upon the experiment. Glycocoll, diglycin, triglycin and leucylglycin exercise some times a favorable effect, phenylglycocoll, on the contrary, acting unfavorably. Heteroalbumose possesses a strong inhibiting action.

8. In the presence of fresh guinea pig's serum, the different protein derivatives are found only exceptionally to exert an accelerating effect, which, moreover is not marked, upon the cobra venom hemolysis of the red corpuscles of the ox and the calf. On the other hand, a strongly inhibiting action is exerted by phenylglycocoll and heteroalbumose and a similar though less marked action is exercised by the larger amounts of diglycin, triglycin and leucylglycin. Triglycin, nevertheless, accelerates

sometimes the hemolysis of the red corpuscles of the calf. The large doses of glycocoll, glycytryptophan, leucin and alanin have a slightly unfavorable action upon the hemolysis of ox blood; such is also sometimes the case with leucin toward the blood of the calf.

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THE RELATION OF THE BRONCHISEPTICIN SKIN REACTION TO IMMUNITY IN CANINE DISTEMPER INCLUDING THE BACTERICIDAL ACTION OF DOG SERUM FOR *B. BRONCHISEPTICUS*¹

ANAPHYLACTIC SKIN REACTIONS IN RELATION TO IMMUNITY. IV.

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In a previous paper (1) we have described the results observed with a cutaneous anaphylactic reaction in canine distemper with a polyvalent emulsion of washed and heat killed *B. bronchisepticus* (Ferry-McGowan). The intracutaneous injection of this emulsion or "bronchisepticin," has been followed by well marked papular or pustular reactions in 77 per cent of dogs suffering with distemper at the time of the tests, and in 60 per cent known to have had the disease while under our observation. Reactions were also observed in 35 per cent of dogs just admitted to the animal house of the University and presenting no clinical evidences of distemper; we were unable to state what percentage of these dogs had had the disease or whether the reactions were purely traumatic. Whether or not *B. bronchisepticus* (Ferry-McGowan) is accepted as the primary etiological agent of canine distemper, we concluded that these anaphylactic reactions may be interpreted as indicating that in this disease a large percentage of dogs display a condition of cutaneous hypersensitiveness to its protein.

The object of the present communication is to record further

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observations with this cutaneous test, and more particularly to study its relation with immunity in canine distemper as part of a series of studies bearing upon the relation of anaphylactic skin reactions to immunity (2, 3 and 4).

METHOD OF STUDY

Bronchisepticin skin tests were conducted among dogs suffering with distemper, those which had had the disease while under our observation and apparently normal dogs. The sera of dogs reacting positively and negatively to bronchisepticin were collected under sterile conditions and subjected to bactericidal, agglutination and complement fixation studies to determine the relation, if any, between the skin reaction on the one hand and the presence or absence of antibodies as detected by tests *in vitro*, on the other. In addition, a number of dogs were kept for a sufficient time under observation to determine whether or not dogs reacting positively to bronchisepticin could contract canine distemper.

The preparation of bronchisepticin and the reactions as observed, have been described in our previous paper (1).

Bactericidal tests were conducted after the pipet method of Wright and a special method which utilizes the dog's own complement, and by the plate method of Stern and Korte.

Agglutination and complement fixation tests were conducted with emulsions of *B. bronchisepticus* and other microorganisms after the technic previously described (2 and 3).

I. Further observations with the bronchisepticin cutaneous reactions

A summary of the results of the intracutaneous bronchisepticin test among 41 dogs is given in table 1.

While the tests are inspected at the end of twenty-four hours, the final readings are not made until forty-eight hours after injection, in order to permit the subsidence of traumatic reactions.

The occurrence of positive reactions among dogs apparently normal and healthy at the time of the tests questions the nature and specificity of the reactions, but it is highly probable that a

TABLE 1

Summary of results with the intracutaneous bronchisepticin test among 41 dogs

CONDITION AT TIME OF TESTS	TOTAL	REACTIONS		PER CENT POSITIVE
		+	-	
Active distemper.....	6	3	3	50.0
Had had distemper.....	15	10	5	67.0
Normal at time of tests.....	20	10	10	50.0

number of these animals had had canine distemper before admission to the animal house.

In order to test the specificity of the reaction to bronchisepticin, a number of dogs were injected at the same time and intracutaneously, with bronchisepticin and an emulsion of typhoid bacilli prepared from the Rawling's strain and administered in the same manner.

The results of these tests are shown in table 2.

TABLE 2

Results of bronchisepticin and typhoidin reaction among dogs and horses

DOG NO.	APPROXIMATE AGE	CONDITION AT TIME OF TESTS	PREVIOUS HISTORY	ALLERGIC REACTIONS WITH BRONCHISEPTICIN		ALLERGIC REACTIONS WITH B. TYPHOUS SUSPENSION	
				Skin	Eye	Skin	Eye
				cm.		cm.	
889	1 yr.	Normal	Distemper	0.8×0.8	—	—	—
682	1½ yrs.	Normal	Normal	1.0×1.5	—	—	—
906	1 yr.	Normal	Normal	1.0×1.5	—	—	—
738	1 yr.	Normal	Distemper	0.6×0.6	—	—	—
657	1 yr.	Normal	Normal	1.5×1.0	—	—	—
664	1 yr.	Normal	Normal	1.5×2.0	—	—	—
783	9 mos.	Normal	Distemper	1.5×1.5	—	—	—
596	9 mos.	Normal	Normal	1.0×2.0	—	—	—
962	1½ yr.	Normal	Normal	—	—	—	—
941	1 yr.	Normal	Normal	—	—	—	—
747	2 yrs.	Normal	Normal	—	—	—	—
718	1½ yrs.	Normal	Normal	—	—	—	—
Horse							
2007	10 yrs.	Normal	Immunized with bronchisepticus	5.0×3.0	—	—	—
2810	8 yrs.	Normal	Normal	2.0×2.0	—	—	—
2833	8 yrs.	Normal	Normal	2.0×2.0	—	—	—

Eight of the twelve dogs reacted in a positive manner to bronchisepticin, but none reacted to the suspension of *B. typhosus*. These tests indicate that the bronchisepticin reactions are not traumatic reactions but apparently true anaphylactic reactions.

Bronchisepticin and typhoidin tests were also conducted among one immunized and two normal horses by injecting 0.1 cc. of the emulsions into the skin of the nose; all reacted to bronchisepticin; horse 2007, however, which had been immunized with *B. bronchisepticus* in the preparation of an immune serum, reacted more strongly than the two normal horses. All reacted negatively to *B. typhosus*.

II. Bactericidal action of dog serum for B. bronchisepticus in relation to the bronchisepticin skin reaction

The fresh sterile sera of 35 dogs reacting positively and negatively to the intracutaneous bronchisepticin test and picked from among animals apparently normal at the time of the tests, dogs sick with distemper and those known to have had the disease and normal dogs after active immunization with a vaccine of *B. bronchisepticus*, were submitted to bactericidal tests after various methods.

The results of 18 tests after Wright's method which employs a constant dose of fresh active serum with varying dilutions of culture, are shown in table 3.

In conducting these tests a twenty-four hour broth culture of *B. bronchisepticus* was employed. Varying dilutions of culture as indicated in table 3 were prepared with sterile broth and the number of living bacilli per cubic centimeter determined by plating with agar. The measure of bactericidal power is expressed according to the approximate number of microorganisms killed. Serum and culture were mixed and incubated at 37°C. for twenty-four hours before culturing in broth.

The results of tests with 15 of the 35 sera examined by the plate method of Stern and Korte, are shown in table 4.

These tests were conducted by mixing in sterile test tubes 0.5 cc. of varying dilutions of sterile inactivated serum, 0.5 cc.

TABLE 3

Results of bronchisepticin and bactericidal tests among dogs apparently normal, dogs suffering with distemper and those known to have had the disease; also among normal dogs after active immunization

NO.	AGE	HISTORY DISTEMPER	ALLERGIC REACTIONS		BACTERICIDAL TESTS*										
			Skin	Eye	1:50 uncont.	1:100 uncont.	1:500 uncont.	1:1000 uncont.	1:5000 uncont.	1:10,000 to 18,000	1:50,000 6000 to 8000	1:100,000 2000 to 3000	1:500,000 3000 to 6000	1:1,000,000 20 to 40	Serum control
					1:50 uncont.	1:100 uncont.	1:500 uncont.	1:1000 uncont.	1:5000 uncont.	1:10,000 to 18,000	1:50,000 6000 to 8000	1:100,000 2000 to 3000	1:500,000 3000 to 6000	1:1,000,000 20 to 40	Serum control
880	1½ years	Normal	0.6×0.8 cm.	+	+	+	+	+	+	+	+	+	+	+	+
909	3 months	Normal	—	—	+	+	+	+	+	+	+	+	+	+	+
829	1 year	Normal	1.0×1.0	—	+	+	+	+	+	+	+	+	+	+	+
682	8 months	At present (3 months)	1.0×1.5	+	+	+	+	+	+	+	+	+	+	+	+
739	6 months	At present (1 month)	—	—	+	+	+	+	+	+	+	+	+	+	+
865	1½ months	At present (3 months)	—	—	+	+	+	+	+	+	+	+	+	+	+
872	1 year	At present (3 months)	—	—	+	+	+	+	+	+	+	+	+	+	+
927	1 year	At present (6 months)	1.0×1.5	—	+	+	+	+	+	+	+	+	+	+	+
891	10 months	At present (1 week)	—	+	+	+	+	+	+	+	+	+	+	+	+
862	1 year	2 months ago	—	—	+	+	+	+	+	+	0	+	+	+	+
809	1 year	1½ months ago	0.5×0.5	+	+	+	+	+	+	+	+	+	+	+	+
897	7 months	2 weeks ago	1.5×1.2	—	+	+	+	+	+	+	+	+	+	+	+
596	10 months	Vaccine	—	—	+	+	+	+	+	+	+	+	+	+	+
597	10 months	Vaccine	—	—	+	+	+	+	+	+	+	+	+	+	+
610	8 months	Vaccine	0.5×0.5	—	+	+	+	+	+	+	+	+	+	+	+
657	10 months	Vaccine	1.0×1.0	—	+	+	+	+	+	+	+	+	+	+	+
637	10 months	Vaccine	—	—	+	+	+	+	+	+	+	+	+	+	+

* By the looped pipet method of Wright.

† + = growth of *B. bronchisepticus*; — = sterile.

TABLE 4
Bactericidal tests after the plate method of Stern and Korte

NO.	HISTORY	SKIN TEST	BACTERICIDAL TESTS.* DILUTIONS OF SERUM									
			1:20	1:30	1:40	1:60	1:80	1:120	1:160	1:240	1:320	Serum control
		cm.										
969	Active distemper	0.6×0.6	+	+	+	+	+	+	+	+	+	-
927	Active distemper	-	+	+	+	+	+	+	+	+	+	-
865	Active distemper	-	+	+	+	+	+	+	+	+	+	-
872	Active distemper	-	+	+	+	+	+	+	+	+	+	-
778	Had distemper	1.0×1.2	+	+	+	+	+	+	+	+	+	-
783	Had distemper	1.5×1.5	+	+	+	+	+	+	+	+	+	-
872	Had distemper	-	+	+	+	+	+	+	+	+	+	-
681	Had distemper	-	+	+	+	+	+	+	+	+	+	-
657	Had vaccine	1.0×1.0	1800	2000	2000	6000	14000	+	+	+	+	-
637	Had vaccine	1.2×0.5	4500	5500	7000	14000	+	+	+	+	+	-
596	Had vaccine	-	4000	6000	10000	+	+	+	+	+	+	-
664	Had vaccine	-	+	+	+	+	+	+	+	+	+	-
778	Normal	1.0×1.2	+	+	+	+	+	+	+	+	+	-
747	Normal	-	+	+	+	+	+	+	+	+	+	-
941	Normal	-	150	900	1200	+	+	+	+	+	+	-

* 0.5 cc. of 1:10 guinea-pig serum complement used in these tests was without appreciable bactericidal effect.

†† = an uncountable number of colonies in the plates.

- = sterile plates.

of sterile guinea-pig serum complement diluted 1:10 with salt solution and 0.5 cc. of a 1:200 dilution of twenty-four hour culture of *B. bronchisepticus*. After incubation at 37°C. for three hours, 10 cc. of agar at 40°C. were added to each tube and poured into sterile petri dishes. Controls on each serum in lowest dilution, complement and culture were included in each experiment.

Plates were counted after 48 hours incubation.

Bactericidal tests were also conducted after a special method consisting of placing in sterile test tubes 0.1, and 0.4 and 0.6 cc. of fresh active serum (not over sixteen hours after collection), 0.1 cc. of a twenty-four hour broth culture of *B. bronchisepticus* and sufficient sterile salt solution to make the total volume in each tube 1 cc. After incubating for three hours, 5 cc.

of broth were added to each tube and re-incubated for forty-eight hours when the results were determined. Serum and culture controls were included in each experiment. The results of a number of tests after this method are shown in table 5.

TABLE 5

Bactericidal tests with 0.1 cc. of twenty-four culture of B. bronchisepticus exposed for three hours to increasing amounts of active serum

NO.	HISTORY	SKIN TEST	AMOUNTS OF ACTIVE SERUM				
			0.1 cc.	0.2 cc.	0.4 cc.	0.6 cc.	Serum control
		cm.					
969	Active distemper	0.6×0.6	+	+	+	+	—
927	Active distemper	—	+	+	—	—	—
865	Active distemper	—	+	+	+	+	—
872	Active distemper	—	+	+	+	—	—
778	Had distemper	1.0×1.2	+	+	+	+	—
783	Had distemper	1.5×1.5	+	—	—	—	—
872	Had distemper	—	+	+	+	—	—
681	Had distemper	—	+	+	—	—	—
685	Had distemper	—	+	+	+	—	—
657	Had vaccine	1.0×1.0	+	+	—	—	—
637	Had vaccine	1.2×1.2	+	+	—	—	—
596	Had vaccine	—	+	+	+	+	—
664	Had vaccine	—	+	+	+	—	—
596	Had vaccine	—	+	+	+	—	—
778	Normal	1.0×1.2	+	+	+	—	—
747	Normal	—	+	+	+	+	—
941	Normal	—	+	+	+	—	—
568	Normal	0.5×0.5	+	—	—	—	—

+ = growth; — = sterility.

SUMMARY OF THE RESULTS OF BACTERICIDAL TESTS

1. The results of bactericidal tests with the same sera varied in the different methods, but as a general rule the bactericidal power of the sera of normal dogs, those suffering with distemper and those having had distemper, is very low for *B. bronchisepticus* as measured by *in vitro* tests.

2. The third method which employed large doses of serum (table 5), showed a bactericidal action of sera which was not apparent in the pipet and plate methods.

3. Normal dog serum may possess a slight bactericidal activity over *B. bronchisepticus* which is apparently not increased during or after an attack of distemper.

4. Active immunization with a vaccine of *B. bronchisepticus* tends to increase the bactericidal power of dog serum for this microorganism.

5. There was no relation between the results of the bronchisepticin skin tests and the bactericidal tests. The sera of those dogs yielding positive skin reactions did not appear to have an appreciable increased bactericidal value.

III. Protection in relation to the bronchisepticin skin test

Of primary importance in the discussion concerning the relation of an allergic skin reaction as an index of immunity, is whether or not a disease is found to occur spontaneously among persons or animals presenting cutaneous hypersensitiveness to the protein of the causative microorganism.

As previously stated a number of our dogs reacted positively to bronchisepticin during the active stages of distemper, while others failed to react after having had the disease.

From April 6, 1916, to June 12, 1916, thirty-three dogs, on all of which the skin tests had been made on the former date, were under our direct observation; two of these which reacted positively to bronchisepticin, contracted distemper during this period. Ten dogs reacting positively and fourteen reacting negatively, did not present clinical evidences of the disease during the same period of observation.

The usual residence of dogs in the animal house is too short to secure a large enough series for determining whether or not ultimately those dogs reacting positively to the bronchisepticin skin tests are immune to distemper or contract the disease; the evidence at hand, however, indicates that a dog reacting positively is still susceptible to canine distemper. Experiments on the possibility of infecting dogs which react positively and negatively with pure cultures of *B. bronchisepticus* are being undertaken to secure more conclusive data.

IV. Agglutination of *B. bronchisepticus* in relation to the bronchisepticin skin reaction

Strains of *B. bronchisepticus* have been found to vary in susceptibility to agglutination; a more complete account of agglutination tests in distemper is given elsewhere (5). Our immediate object in this portion of the investigation was to determine the relation, if any, between the bronchisepticin skin tests and the presence or absence of agglutinins for *B. bronchisepticus* in dog serum. Since agglutinins are regarded by many as intimately concerned in the phenomenon of bacteriolysis, we have summarized the results of bronchisepticin, agglutination and bacteriolytic tests² (shown in tables 3, 4, 5, 6, 7, 8 and 9) as follows:

1. Normal dog serum may agglutinate *B. bronchisepticus* in dilutions as high as 1:40. During the acute stages of distemper the agglutination titer may reach 1:160 and drop to 1:80 or normal in a comparatively short time after the subsidence of the disease. Active immunization increases the agglutinin content. As a general rule, however, it may be stated that the agglutinins for *B. bronchisepticus* are not greatly increased in distemper and agglutination tests have been found of no value in the diagnosis of the disease.

2. As previously stated the bactericidal value of normal and distemperous dog serum for *B. bronchisepticus* is very slight, and only appreciable under conditions where the dog's own complements are utilized, when the proportion of serum is in excess of the culture. Dog sera which agglutinate *B. bronchisepticus* in dilutions up to 1:40 or higher, may show no appreciable bactericidal activity; in our experiments we were unable to detect a relationship between the agglutinins and bacteriolysins, but this may be due to the low content of both and insufficient delicacy of tests *in vitro* for these antibodies.

3. Our experiments demonstrate quite definitely, however, that there is no relation between the skin tests and the presence of agglutinins in the sera. Positive skin reactions have occurred

²Most of the agglutination and bacteriolytic tests were conducted with a single strain of *B. bronchisepticus*.

in dogs whose sera were high or low in agglutinins and vice versa without a demonstrable relationship between the two reactions.

V. Complement fixation in relation to the bronchisepticin skin reactions

Complement fixation tests were conducted with sera of normal and distemperous dogs to determine if there was any relation between the skin tests and the presence or absence of complement fixing antibody. Unfortunately normal dog serum is prone to yield non-specific reactions and particularly with bacterial antigens (6), so that it is extremely hazardous to draw conclusions on the basis of complement fixation tests. As pointed out elsewhere (5) complement fixation reactions have been found of little practical value in the diagnosis of canine distemper and this is largely due to the tendency of normal dog sera to yield non-specific reactions that cannot be differentiated from true complement fixation by specific antibody.

Complement fixation tests were conducted with various polyvalent antigens of *B. bronchisepticus* and of the staphylococci and streptococci so frequently found in the upper respiratory passages of distemperous dogs. These antigens were prepared by cultivating the microorganisms on a solid medium, washing once or twice by centrifugalization, re-suspending in normal salt solution and heating at 60°C. for one hour. In all experiments the antigens were titrated preliminary to the tests and were used in doses corresponding to one-third or one-quarter the anticomplementary dose. In the titrations and complement fixation tests, at least two units of hemolysin were employed to overcome, to some extent, non-specific complement fixation; for the same reason all sera were tested while fresh and unheated. The results are shown in tables 6, 7, 8 and 9.

The occurrence of positive reactions and particularly the degree of reaction, were less among our so-called normal dogs (table 6) than those suffering with distemper (table 7) or known to have had the disease (table 8) or having received bronchisepticus vaccine (table 9). No relation was found, however,

TABLE 6

Results of agglutination, complement fixation and bronchisepticin skin tests among normal dogs

NO.	AGE	ALLERGIC REACTIONS		AGGLUT.	COMPLEMENT FIXATION TESTS*					
		Skin	Eye		1 B. bronchi.	2 B. bronchi.	3 B. bronchi.	4 strept.	5 staph.	Serum control
		cm.								
909	3 months	—	—	1:20	—	—	—	—	—	—
828	1 year	1.0×1.0	—	1:20	—	+	—	—	++	—
853	1 year.	1.0×1.0	—	1:20	++	++	++	+	++	—
880	1½ years	0.6×0.8	—	1:40	—	—	—	—	—	—
941	1 year	—	—	1:20	++	—	—	—	—	—
747	1½ years	—	—	1:10	++	++	++	+	++	—
568	1 year	0.5×0.5	—	1:40	++	++	+++	+	++++	—

* Conducted with active serum in dose of 0.1 cc.

between the skin reactions and the complement fixation tests, although in similar studies in other diseases positive complement fixation tests have usually occurred with the sera of persons yielding positive skin reactions (2, 3, and 4).

CONCLUSIONS

1. Anaphylactic skin tests with a polyvalent antigen of washed and heat killed *B. bronchisepticus* (Ferry-McGowan) indicate that a large percentage of dogs are hypersensitive to the protein of this bacillus.

2. Dogs known to have had distemper yielded the highest percentage of positive reactions; a large proportion of apparently normal dogs reacted in a positive manner when tested soon after admission to the animal house, but there was no means of learning what proportion of these animals had had the disease.

3. Bronchisepticin intracutaneous reactions are probably specific reactions due to hypersensitiveness to the protein of *B. bronchisepticus*; similar tests with an emulsion of typhoid bacilli yielded negative reactions.

4. The sera of normal dogs, those suffering with distemper and those convalescent from this infection, possess slight or

TABLE 7
Results of agglutination, complement-fixation and bronchiseptin skin tests among dogs suffering with distemper

NO.	AGE	HISTORY OF DISTEMPER (DURATION)	ALLERGIC REACTION		AGGLU- TINA- TION	COMPLETE FIXATION TESTS*					Serum control
			Skin	Eye		1 B. bronchi.	2 B. bronchi.	3 B. bronchi.	4 strept.	5 staph.	
			cm.								
682	8 months	3 months	1.0×1.5	—	1:40	+++	+++	+++	+++	+++	—
739	6 months	1 month	—	—	1:40	+++	+++	+++	+++	—	—
891	10 months	1 week	—	—	1:40	++	++	+	—	—	—
681	6 months	10 weeks	0.8×0.8	—	1:40	+	+	—	—	—	—
568	1½ years	3 months	—	—	1:160	+++	+++	+++	+++	+++	—
872	1 year	3 months	—	—	1:80	++	++	+	—	+	—
927	1 year	6½ months	—	—	1:80	+	+	+	—	—	—

* Conducted with active serum (0.1 cc.)

TABLE 8
Results of agglutination, complement fixation and bronchiseptin skin tests among dogs known to have had distemper.

NO.	AGE	HAD DISTEMPER	ALLERGIC REACTION		AGGLU- TINA- TION	COMPLETE FIXATION TESTS*					Serum control
			Skin	Eye		1 B. bronchi.	2 B. bronchi.	3 B. bronchi.	4 strept.	5 staph.	
			cm.								
862	1 year	2 months ago	—	—	1:40	+++	+++	+++	—	+++	—
809	1 year	1½ months ago	0.5×0.5	+	1:20	±	±	±	—	±	—
879	7 months	2 weeks ago	1.5×1.2	+	1:20	++	++	++	++	+++	—
829	1 year	1 month ago	—	—	1:40	+++	+++	+++	—	+++	—
862	10 months	1 month ago	1.0×1.0	—	1:20	—	—	—	—	—	—
685	1½ years	5 months ago	—	—	1:80	+++	+++	+++	+++	+++	—
710	2 years	4½ months ago	—	—	1:40	—	—	—	—	—	—
681	10 months	5 months ago	—	—	1:20	++	++	++	—	++	—
778	1 year	4 months ago	1.0×1.2	—	1:20	+++	+++	+++	+++	+++	—

* Conducted with active serum (0.1 cc.)

TABLE 9
Results of agglutination, complement-fixation and bronchisepticin skin tests among normal dogs after active immunization with *B. bronchisepticus* vaccine

No.	AGE	ALLERGIC REACTIONS		AGGLUTINATION	COMPLEMENT FIXATION					Serum control
		Skin	Eye		1 <i>B.</i> bronchi	2 <i>B.</i> bronchi	3 <i>B.</i> bronchi	4 strept.	5 staph.	
		cm.								
596	10 months	—	—	1:20	0	0	0	0	0	0
597	10 months	—	—	1:80	++	++	++	++	++	—
610	8 months	0.5×0.5	+	1:20	+	+	+	+	+	—
596	10 months	—	—	1:10	++	++	++	++	++	—
657	10 months	1.0×1.0	—	1:40	0	0	0	0	0	0
637	10 months	0.5×0.2	++	1:80	++	++	++	++	++	—
664	9 months	1.0×1.2	+	1:40	0	0	0	0	0	0
596	10 months	—	—	1:80	++	++	++	—	++	—
665	8 months	—	—	1:20	+	+	+	—	—	—
675	8 months	—	—	1:80	++	++	++	+	+	—
657	10 months	1.0×1.0	—	1:20	±	±	±	—	—	—
639	8 months	—	—	1:40	++	++	++	++	++	—
671	8 months	0.4×0.6	—	1:40	++	++	++	++	++	—

no bactericidal activity over *B. bronchisepticus* as measured by tests *in vitro*.

5. There is no relation between the results of bronchisepticin anaphylactic reactions and the bactericidal power of the serum. The sera of dogs yielding positive anaphylactic reactions in the skin did not appear to have an appreciable increased bactericidal value.

6. Agglutinins and complement fixing antibodies for *B. bronchisepticus* appear to be increased to some extent in distemper. Normal dog serum contains agglutinin for this bacillus and yields non-specific complement fixation reactions so that both procedures possess but little practical value in the diagnosis of distemper. In our experiments we were unable to detect a relationship between the agglutinins and bacteriolysins on the one hand and the skin reactions on the other.

7. Evidence at hand indicates that dogs yielding positive bronchisepticin reactions are still susceptible to canine distemper.

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